Volume 28 May 15, 1954 Number 4

IOWA STATE COLLEGE JOURNAL OF SCIENCE

A Quarterly of Research

CONTENTS

PUBLISHED BY
THE IOWA STATE COLLEGE PRESS
PRESS BUILDING
AMES, IOWA

IOWA STATE COLLEGE

JOURNAL OF SCIENCE

Published August, November, February, and May

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Entered as second-class matter January 16, 1935, at the post office at Ames, Iowa, under the act of March 3, 1879.

IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 461-465

THE NATURE OF THE SPARING PHENOMENON. IV. THE ROLE OF COMPLEMENT IN THE HEMAGGLUTININ-INHIBITION PHENOMENON*

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Duck plasma possesses the power of inhibiting to a greater or lesser degree the agglutination of duck erythrocytes by normal chicken plasmas (2). The inhibition was observed in 28 of 29 frozen and thawed plasmas that exhibited native ability to agglutinate duck erythrocytes (3). The possible role of complement in the observed hemagglutination-inhibition has not thus far been reported.

MATERIALS AND METHODS

The techniques employed in making the in vitro hemagglutination and hemagglutination-inhibition tests have been described previously (2, 3). The hemagglutination systems consisted of dilutions of chicken plasma or serum that agglutinated duck erythrocytes, tested against various dilutions of duck plasma or serum to determine their hemagglutinin-inhibiting effect.

The chicken plasmas and sera used were innately capable of agglutinating duck erythrocytes. Unless otherwise stated, they had been frozen for several days and thawed before use. This statement is pertinent because Schwink and Becker (8) found that freezing in the refrigerator may increase the titer of isohemagglutinin in chick plasma, and Becker, Schwink and Probst (3) observed a similar effect of freezing certain chicken plasmas on agglutination of duck erythrocytes.

It is now known that complement has the following components (4, pp. 117-126): C_1', which is heat-labile and precipitated by dialyzing against water; C_2', which is heat-labile and soluble in water during dialysis; C_3', which is heat-stable, precipitated by dialysis, and destroyed during contact with live yeast or zymosan; and C_4', which is heat-stable, soluble in water during dialysis, and destroyed by ammonia. C_1' and C_3' together constitute the midpiece (M), while C_2' and C_4' constitute the endpiece (E). The yeast- or zymosan-treated fraction has been called Z; the ammonia-treated fraction, N. According to Pillemer, Seifter and Ecker (7), the endpiece is relatively acid-labile and alkali-stable, while the midpiece is acid-stable and alkali-labile. Complement is inactivated by pH above 9.5 and pH below 4.2.

To insure that the possible role of each component of duck plasma or serum in the sparing phenomenon was adequately explored, duck plasma, and serum, were subjected to the following treatments:

*This investigation was supported (in part) by a grant from the Industrial Science Research Institute, Iowa State College.

1. Heating at 56°C for 20 min. or 30 min. in order to destroy C_1' an C_2'.

2. Incubating with 3 g. of fresh Fleishmann's yeast at 37°C for tw hours, shaking a mixture of 10 cc. of plasma every 15 minutes to destro C_3'. After two hours, the mixture was made up to 20 cc. with physiologi cal salt solution and centrifuged for 30 minutes at 2500 rpm. The super nate was used in the tests. (5)

3. Treating with ammonia in order to destroy C_4'. Eight cc. of duc plasma were diluted with two cc. of N/10 NH_4OH, incubated at 37°C fo two hours, adjusted to pH 7.0 with N/10 HCl and filtered through filte paper. (6)

4. Treating with acid to destroy the endpiece ($E = C_2' + C_4'$). Duc plasma was diluted with N/10 HCl to pH 4.2, incubated at 37°C for tw hours, adjusted to pH 7.0 with N/10 NaOH, and filtered through filte paper.

5. Treating with alkali to destroy the midpiece ($M = C_1' + C_3'$). Duc plasma was diluted with N/10 NaOH to pH 9.5, incubated at 37°C for tw hours, the mixture adjusted to pH 7.0 with N/10 HCl and filtered throug filter paper.

It seems fairly certain that, if any of the components were concerne in the role of the duck plasma hemagglutinin-inhibition phenomenon, th effect of their inactivation would have been observed in these tests.

RESULTS

Effects of heating the duck plasma. A previous contribution reporte (2) that heating chicken plasma used at 56°C for 35 min. did not affec agglutinating titers, and that heating both the chicken plasma and the duc plasma used in the system at 56°C for 30 min. or 40 min. yielded the same result as when either or both were heated.

More recently duck or chicken plasma or serum, or both, have bee heated in the range from 56° - 65°C for 20 to 30 minutes. Clear serum from the clot proved better then plasma, because heating caused consider able clouding in the latter. Centrifugation at 2500 rpm for 20 minutes, however, largely cleared plasma of insoluble material (fibrinogen).

One test especially worthy of mention involved dilutions of three pre viously frozen and thawed chicken plasmas that agglutinated duck erythro cytes used against fresh and unfrozen duck serum capable of inhibiting hemagglutination in various degrees, according to its dilution. The read ings shown in Table 1 indicate that the hemagglutinin-inhibiting property of the unheated duck serum was the same at all the dilutions tested as that of the serum heated at 63°C for 25 min.

Effects of incubating with fresh yeast. Pillemer and Ecker (5) demon strated an anticomplementary factor in fresh yeast by incubating serum with either fresh yeast or an extract of the yeast. It was our intention to prepare the extract containing zymosan if positive results were obtained with the whole yeast cells; but our negative results rendered it unnecessary.

Experiments were made with seven previously frozen chick plasmas that agglutinated chicken erythrocytes. In each of them the inhibiting ef fect of previously frozen duck plasma was compared with that of the same duck plasma incubated with yeast.

Table 1. Degree of hemagglutination in systems containing heated or unheated duck serum as hemagglutinin-inhibitors.

uck rbc uspension	Plasma	0.85 per cent salt solution	D. serum unheated		D. serum heated		Read-ing
cc.	cc.	cc.	dil.	amt.	dil.	amt.	
	Chick 30						
0.2	0.2	0.5	1/8	0.1			-x
0.2	0.2	0.5			1/8	0.1	-x
0.2	0.2	0.6					3x
0.2	0.2	0.5	1/16	0.1			+x
0.2	0.2	0.5			1/16	0.1	+x
0.2	0.2	0.5	1/32	0.1			+2x
0.2	0.2	0.5			1/32	0.1	+2x
	Chick 80						
0.2	0.1	0.6	1/8	0.1			$\frac{+}{-}$
0.2	0.1	0.6			1/8	0.1	$\frac{+}{-}$
0.2	0.1	0.7					$\frac{3x}{-}$
0.2	0.1	0.6	1/16	0.1			+
0.2	0.1	0.6			1/16	0.1	+
0.2	0.1	0.6	1/32	0.1			x
0.2	0.1	0.6			1/32	0.1	x
	Chick 113						
0.2	0.2	0.5	1/16	0.1			-x
0.2	0.2	0.5			1/16	0.1	-x
0.2	0.2	0.6					3x
0.2	0.2	0.5	1/32	0.1			+x
0.2	0.2	0.5			1/32	0.1	+x
0.2	0.2	0.5	1/64	0.1			2x
0.2	0.2	0.5			1/64	0.1	2x

The amounts of chicken plasma employed in each test was either 0.1 or 0.2 cc., and the dilution of the duck plasma in each system ranged from 1:40-1:1280. In every test the readings for the inhibiting effects of control duck plasmas were the same as for the yeast-treated plasmas.

Effect of ammonia. Eight series of tests similar to the above were made, each with a chicken plasma that agglutinated duck erythrocytes. In each test the inhibiting duck plasma was tested untreated and after incubation with ammonia. In seven of the series the observed inhibiting effects of the various dilutions of duck plasma were comparable in the controls and in the tests. In one series there was but a shade of difference between the inhibiting effects at 1:320 and 1:640 dilutions of the duck plasma, with readings of x and 2x, respectively, for the controls, and +x and +2x, respectively, for the tests.

Effect of acid treatment. Adjustment of the reaction of the duck plasma to 4.2 with HCl and incubation for two hours produced very little, if any, change in the inhibiting property of duck plasma for agglutination of duck erythrocytes by seven different hemagglutinating chicken plasmas. The readings for the controls and tests were practically identical, any differences being of about the same order as in the preceding experiment.

Effect of alkali. The inhibitory effects of the untreated duck plasma and plasma adjusted to pH 9.5 with NaOH and incubated for two hours were practically uniform in tests with seven hemagglutinating chicken plasmas. The differences noted were minor, as in the two preceding experiments.

DISCUSSION

In testing a hypothesis to the effect that complement has a role in hemagglutination-inhibiting by duck plasma or serum in an agglutinating system involving chicken plasma or serum and duck erythrocytes, it was necessary to take into consideration the fact that complement was known to possess at least four components, C_1', C_2', C_3', and C_4', each with distinctive properties. Heat-treatment of duck plasma should have inactivated its inhibiting effect by virtue of destruction of C_1' and C_2'. Treatment with yeast and NH_3^+ should have inactivated it by destroying C_3' and C_4', respectively. Even granting that the chicken plasma itself might have supplied some of the destroyed factors, it could not have done so for all of them, because hemagglutinin-inhibition is an effect conferred by supplements of duck plasma.

It appears safe to declare definitively that complement does not play a role in the hemagglutinin-inhibition phenomenon. As stated in previous reports, the phenomenon is to be attributed to a nonadsorbable, comparatively thermostable, cryostable component of duck plasma. The responsible substance has been shown to be a seromucoid (3).

It appears also that the hemagglutinin-inhibiting and sparing effects (1) are due to seromucoid reacting with the antibody (agglutinin) instead of preëmpting positions on the erythrocyte (antigen) which would otherwise have been assumed by the antibody. Thus the two effects are not explainable by seromucoid playing the role of "blocking antibody", at least in the conventional sense. As we conceive it, the role of the inhibiting principle, seromucoid, is much more like that of antigen than of antibody.

CONCLUSION

Complement is not concerned in the role that duck plasma plays in inhibiting the hemagglutination of duck erythrocytes by chicken plasma.

REFERENCES

1. Becker, E.R. and T.M. Schwink. The nature of the sparing phenomenon. II. Activity in vivo and nature of the sparing substance. Jour. Inf. Dis. 92:74-76. 1953.
2. _____, _____ and R.M. Prather. Hemagglutinin-inhibiting property of duck plasma exhibited in agglutination reactions involving duck

erythrocytes and plasmas of chicks recovered from lophurae malaria. Jour. Inf. Dis. 89:95-102. 1951.

. _____, _____ and R.T. Probst. The nature of the sparing phenomenon. I. Activity and nature of hemagglutinin-inhibitor. Iowa State College Jour. Sci. 27:79-90. 1952.

. Kabat, E.A., and M. M. Mayer. Experimental Immunochemistry. C.C. Thomas, Springfield, Ill. 1948.

. Pillemer, L., and E.E. Ecker. Anticomplementary factor in fresh yeast. Jour. Biol. Chem. 137:139-142. 1941.

. _____, J. Seifter, and E.E. Ecker. The effect of amino compounds on the fourth component of complement. Jour. Immunol. 40:89-99. 1941.

. _____, _____, and _____. The effect of acids and alkalis on the fourth component of complement, and the role of calcium. Jour. Immunol. 40:101-106. 1941.

8. Schwink, T.M., and E.R. Becker. Studies in chicken isohemagglutinins and their relation to passive immunity in P. lophurae malaria. Jour. Parasit. 37(5, Sec. 2):11-12. 1951.

IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 467-475

DISTRIBUTION IN THE YOUNG DAIRY CALF OF ORALLY ADMINISTERED P^{32} *

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In recent years P_{32} has been used extensively as a radioactive indicator in the study of phosphorus metabolism. Preliminary to more intensive investigations in the bovine of specific biological reactions involving phosphorus-containing compounds, a study of the in vivo fate of the radioactive tracer seemed desirable. Therefore, the present study was undertaken to characterize chronologically the changes in quantity and type of phosphorus compounds in the blood following oral administration of P^{32} and to determine the distribution of the isotope in the tissues of the young dairy calf.

EXPERIMENTAL

The experimental animals, two male Jersey calves from the Iowa State College dairy herd, were placed in a stall in an air-conditioned room maintained at a temperature of $68^{\circ} \pm 1^{\circ}$ F. The stall was constructed so as to allow the separate collection of urine and feces. The animals were maintained on a whole milk diet. Pertinent data relative to the experimental animals are presented in Table 1. The radioactive phosphorus ($H_3P^{32}O_4$ in 0.014 N HCl) was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, and was reported as 99.9 per cent pure.

TABLE 1

Summary of data relative to experimental animals and treatments

Calf no.	Age at start of experiment (days)	Weight (kg.)	P^{32} administered (mc)	Duration of experiment (hours)
1	19	27.3	5.15	148
2	5	34.1	2.23	76

Venous blood samples (heparin anticoagulant) were collected several times during the 24 hours subsequent to the administration of the isotope, and at less frequent intervals thereafter. Small portions of the whole

* Journal Paper No. J-2305 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 814.

blood and of plasma were taken for analysis. Lipid phosphorus was separated from aliquots of plasma by precipitation with 10 per cent trichloroacetic acid according to the method of Zilversmit and Davis (13). The filtrate, after removal of protein and lipid phosphorus, was employed for total acid-soluble phosphorus and inorganic phosphate determinations, the latter being precipitated with strychnine molybdate according to the procedure of Tisdall (11). All samples then were stored in a refrigerator until assayed for P^{32} activity.

Following sacrifice of the animals, the desired organs and tissues were excised and samples were saved for analysis. In preparation for the measurement of radioactivity, weighed portions of tissues were dried at 100°C. with 10 per cent magnesium nitrate, and then were ashed at 600-700°C. in an electric muffle furnace. The ashed samples were dissolved in dilute hydrochloric acid and stored for subsequent analysis.

Samples of several intestinal tissues were homogenized in a Waring blender. Subsequently, the trichloroacetic acid precipitable, total acid-soluble, and inorganic phosphorus fractions were prepared in a manner similar to that described above for blood plasma. In addition, ingesta from various segments of the digestive tract were taken for P^{32} analysis.

The aforementioned aliquots of whole blood, blood plasma, blood phosphorus fractions, tissue fractions and ingesta were then dried on 2-inch watch glasses and assayed for P^{32} activity with a thin-walled Geiger-Mueller counting tube employing a Model 161 Scaling Unit.* A minimum of 5 minutes was allowed for counting each sample. All samples were counted in triplicate and the values were averaged to decrease the error due to differences in geometry. Backgrounds and decay corrections were applied to all determinations.

The phosphorus content in the various samples was determined colorimetrically using a Klett-Summerson photoelectric colorimeter with filter 66. Lipid phosphorus was estimated by the method of Zilversmit and Davis (13). Total, acid-soluble, and inorganic phosphorus values were determined by the method of Fiske and SubbaRow (5). The organic acid-soluble phosphorus fraction was obtained by subtracting the inorganic phosphorus from the total acid-soluble phosphorus.

Radioautographs were made of a tibia from each animal. These bones were cut in half longitudinally, placed in contact with commercial Ortho film, wrapped tightly in dark paper to exclude light, and left the required time for exposure (estimated by the method of Yagota (12)).

RESULTS

The distribution (calf 1) of radioactive phosphorus in the whole blood, plasma and cells is presented in Fig. 1. The data show that the P^{32} activity rapidly appeared in the plasma and was incorporated into the cells at a slower rate. After about 36 hours the radioactivity in the cells exceeded that in the plasma.

Fig. 2 shows the distribution of the P^{32} activity in the blood plasma of calf 1. The activity initially was present largely in the inorganic phosphate fraction of the blood plasma, but after approximately 10 hours th

* Nuclear Instrument and Chemical Corporation.

najor portion of the activity was in the lipid fraction. A small but signi-
icant quantity of P^{32} appeared in the organic acid-soluble phosphorus
raction. The data obtained with calf 2 presented essentially the same
eneral trends but were not so complete because of the smaller dose of
he isotope and the shorter experimental period.

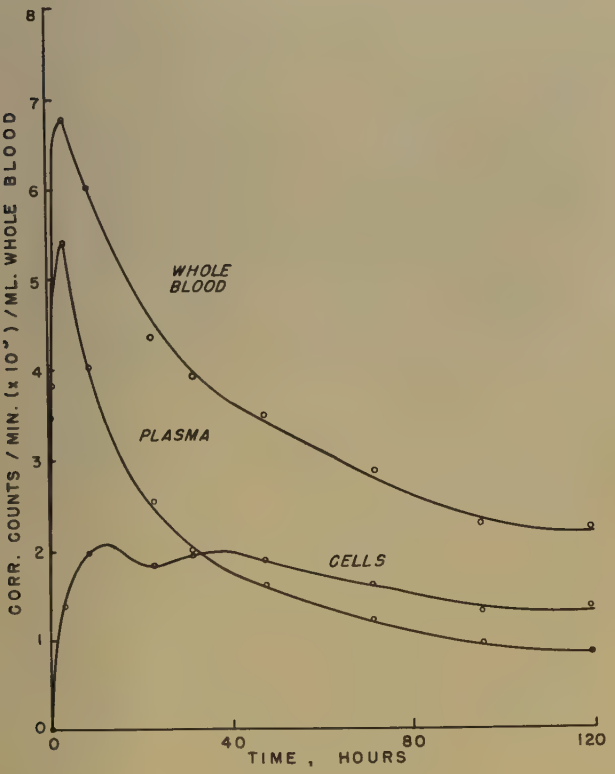

Fig. 1. Changes in P^{32} activity in whole blood, plasma and cells following oral administration of $H_3P^{32}O_4$.

The total urinary excretion of P^{32} from calf 1 was 9.1 per cent of the administered isotope, while that from calf 2 was 8.2 per cent; the former covered a 6-day period, the latter a 3-day period. These results are in close agreement with the work of Erf, Tuttle and Lawrence (3) on the urinary excretion of P^{32} by humans.

Results of the distribution of P^{32} in the two animals were not strictly analogous due to differences in age and weight of the animals, in duration of the trials and in P^{32} dosages. To correct for differences in weight and dosage, calculation of the distribution was made on the basis of standard specific activity which is defined by Smith and associates (10) as:

$$\frac{\text{microcuries } P^{32}/\text{mg. P}}{\text{millicuries } P^{32} \text{ fed/kg. body weight}}$$

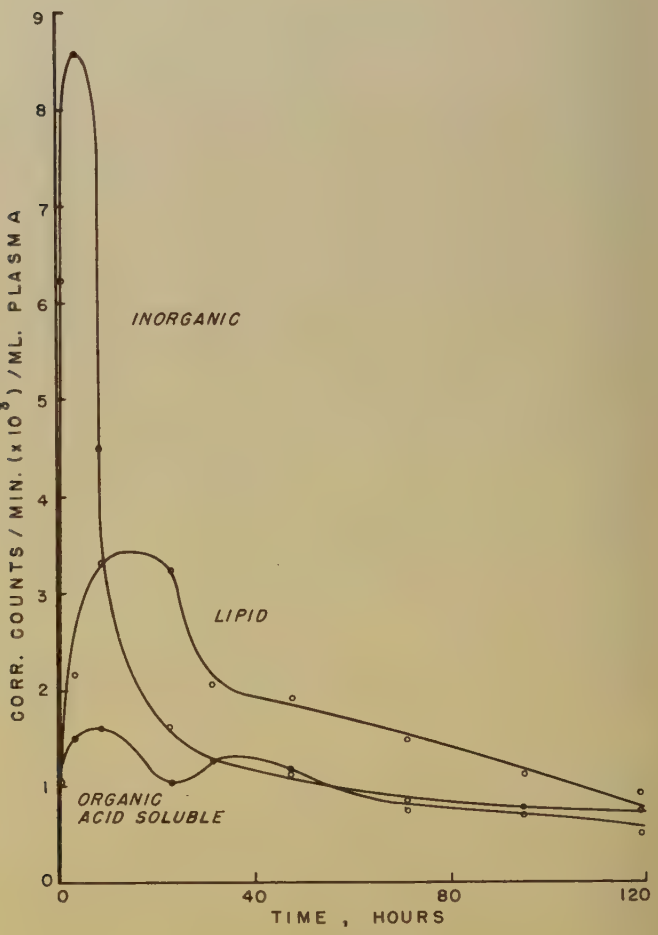

Fig. 2. Changes in P^{32} activity in the blood plasma phosphorus fractions following oral administration of $H_3P^{32}O_4$.

The distribution of P^{32}, expressed as standard specific activity, in various tissues of the two calves is shown in Fig. 3. The data indicate that the intestinal tissues and the various organs tended to have the highest values, whereas the skeletal structure contained smaller proportions of P^{32}.

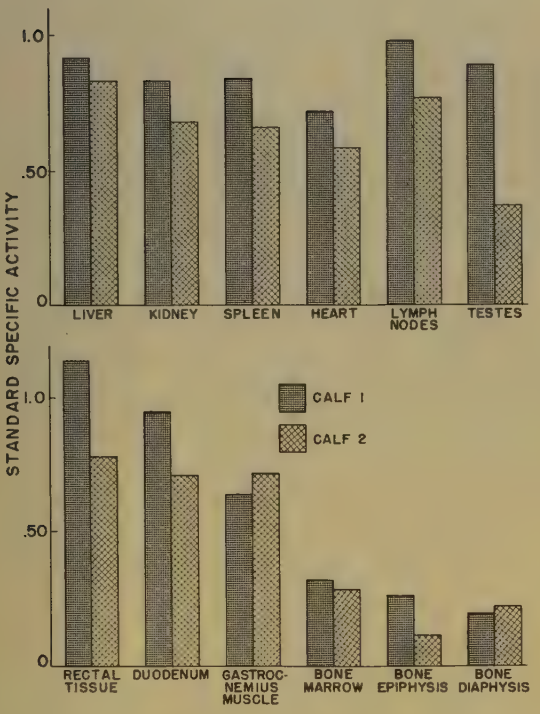

Fig. 3. Standard specific activity of P^{32} in several tissues.

The radioactivity of the various phosphorus fractions in the intestinal tissues and in the ingesta of these sections is summarized in Table 2. The data indicate that the levels of trichloroacetic acid precipitable P^{32} and of inorganic P^{32} in tissue samples from the small intestine and rectum

tend to be higher than the levels of the acid-soluble organic P^{32}. It also is apparent that a considerable quantity of radioactivity remained in, or had been re-excreted into, the ingesta of the gastro-intestinal tract. The greater values obtained with calf 2 probably were due to the fact that the animal did not defecate during the experiment.

TABLE 2

P^{32} activity in homogenized tissues and digestive tract ingesta

Calf	Tissue	Corr. counts/min./g. fresh tissue			Corr. counts/ min./g. fresh ingesta
		TCA P[a]	Inorganic P	Organic acid-sol. P	
1	Duodenum	10770 ± 41[b]	3840 ± 42	3910 ± 26	8940 ± 27
	Ileum	22200 ± 68	15900 ± 86	7960 ± 43	12900 ± 65
	Rectum	14960 ± 59	15140 ± 86	2160 ± 15	7340 ± 27
2	Duodenum	1320 ± 9	1810 ± 15	905 ± 8	50200 ± 280
	Jejunum	1680 ± 12	2890 ± 23	715 ± 6	51200 ± 350
	Rectum	2940 ± 21	4120 ± 36	1330 ± 12	12400 ± 67

[a] Trichloroacetic acid (TCA) precipitable phosphorus which includes lipid and tissue protein phosphorus.

[b] Counts ± standard deviation.

Radioautographs of longitudinal sections of tibias (one from each calf) are shown in Fig. 4 and demonstrate that a larger quantity of P^{32} was deposited in the epiphysis than in the diaphysis. On the basis of total counts per gram of dry tissue the P^{32} activity in the bone epiphysis exceeded that of all tissues in the calves. However, due to the high total phosphorus content of the epiphysis, the value for the standard specific activity (Fig. 3) was less than that for several of the other organs and tissues.

DISCUSSION

For a short time following the oral administration of P^{32} to the calves, the activity in the blood was found to be concentrated largely in the plasma inorganic phosphorus fraction. However, after a few hours, the highest concentration of the isotope was found in the cells and in the lipid phosphorus fraction of the blood plasma, showing that inorganic phosphate was being utilized in the synthesis of organic phosphorus compounds. The trends in plasma inorganic P^{32} and phospholipid P^{32} are in agreement with the findings of Barker, Rogers and Moore (1) who reported similar changes in dogs fed the isotope. The present data, however, do not agree closely with those of Saarinen and associates (9) who found two activity maxima in both whole blood and blood plasma (one at 5-6 hours and the other at about 30 hours), following oral administration of inorganic P^{32} to a lactating dairy cow. These workers also reported that the blood plasma

hospholipid P^{32} values did not increase appreciably until approximately 4 hours after feeding the isotope. Since the calves were essentially non-ruminating animals and since the cow was lactating, close agreement in ne above data probably should not be expected.

Fig. 4. Radioautographs of longitudinal sections of tibias from calf 1 (upper) and calf 2 (lower).

Since in the present study relative large amounts of P^{32} were incorporated into phosphatides, the site of phosphatide synthesis is of considerable interest. Fishler and co-workers (4), who studied the phosphatide turnover in hepatectomized dogs which had been fed radioactive phosphorus, reported recovery of labeled phosphatides from the kidney and small intestine, indicating that the liver is not the sole source of phosphatide synthesis. These workers also demonstrated that transfer of phospholipids from kidney and small intestine to the plasma occurred at a relatively slow rate. Zilversmit and DiLuzio (14) found that phospholipid synthesis in dogs occurred primarily in the liver, with smaller amounts being produced in the kidney and small intestine. That the liver is the principal organ wherein phospholipid synthesis occurs in dogs has been demonstrated further by the work of Fischler et al. (4) and of Entenman, Chaikoff and Zilversmit (2). It seems logical, therefore, to assume that the rather high levels of phospholipid P^{32} in the intestinal tissues observed in the present study originated primarily in the liver.

The distribution of P^{32} in the various tissues and organs indicates that the standard specific activities of the gastro-intestinal tissues, lymph nodes, liver, heart, kidney, testes, and spleen were relatively high.

Lower proportions were found in the skeletal muscle and bone. Similar results have been reported by Lofgreen, Kleiber and Smith (7) who studied the absorption and distribution of orally administered P^{32} labeled casein in young calves. Smith and associates (10) studied the distribution of injected $Na_3P^{32}O_4$ in swine tissues and, although age affected the order of distribution, the results observed were similar to those for the calves in the present study.

Since a considerable amount of activity was found in the intestinal ingesta in calf 1, re-excretion of P^{32} probably occurred. This is in agreement with the work of Lofgreen, Kleiber and Smith (8) who found that all segments of the gastro-intestinal tract may be involved in the excretion of phosphorus. These workers believe that the jejunum plays a major role in this respect. The type of phosphorus in the ingesta was not determined in the present study, but it seems unlikely that much of the administered inorganic P^{32} could have been present in the ingesta after a period of 6 days.

A considerable quantity of P^{32} was found in the bone of the young calf. This might be expected since the bones in these animals are rapidly growing structures. Moreover, it has been suggested by Hevesy (6) that the P^{32} uptake of bones is due primarily to an almost irreversible incorporation of the isotope with the bone apatite crystals.

SUMMARY

Inorganic P^{32} administered orally to the young calf was found initially in the plasma inorganic phosphorus fraction of the blood, but gradually concentrated in the blood cells and plasma lipid fractions. Subsequent to attainment of maxima the P^{32} values declined in all fractions.

Tissues with high standard specific activity included the gastro-intestinal tissues, lymph nodes, liver, heart, kidney, testes, and spleen. Lower concentrations were found in skeletal muscle and bone.

The use of radioautographs has shown that P^{32} is deposited in the developing bone, particularly in the epiphysis.

REFERENCES

1. Barker, W.F., K.E. Rogers, and F.D. Moore. Effect of pancreatectomy on phospholipid synthesis in the dog. Arch. Surgery, 61:1151. 1950.
2. Entenman, C., I.L. Chaikoff, and D.B. Zilversmit. Removal of plasma phospholipids as a function of the liver: The effect of exclusion of the liver on the turnover rate of plasma phospholipids as measured with radioactive phosphorus. Jour. Biol. Chem. 166:15. 1946.
3. Erf, L.A., L.W. Tuttle, and J.H. Lawrence. Clinical studies with the aid of radiophosphorus. IV. The retention in blood, the excretion and the therapeutic effect of radiophosphorus on patients with leukemia. Ann. Internal Med., 15:487. 1941.
4. Fishler, M.C., C. Entenman, M.L. Montgomery, and I.L. Chaikoff. The formation of phospholipid by the hepatectomized dog as measured with radioactive phosphorus. I. The site of formation of plasma phospholipids. J. Biol. Chem. 150:47. 1943.

5. Fiske, C.H., and Y. SubbaRow. The colorimetric determination of phosphorus. Jour. Biol. Chem. 66:375. 1925.
6. Hevesy, G. Radioactive Indicators. p 417. Interscience Publishers, Inc., New York. 1948.
7. Lofgreen, G.P., M. Kleiber, and A.H. Smith. The digestion and absorption of P^{32} labeled casein by the young calf. Jour. Nutr. 43: 401. 1951.
8. _____, _____, and _____. The excretion of injected P^{32} into the gastrointestinal tract of the young calf. Jour. Nutr. 47:561. 1952.
9. Saarinen, P., C.L. Comar, S.P. Marshall, and G.K. Davis. Partition of orally administered radioactive phosphorus in the blood and milk of the dairy cow. Jour. Dairy Sci. 33:878. 1950.
10. Smith, A.H., M. Kleiber, A.L. Black, M. Edick, R.R. Robinson, and H. Heitman, Jr. Distribution of intravenously injected radioactive phosphorus (P^{32}) among swine tissues. Jour. Ani. Sci. 10: 893. 1951.
11. Tisdall, F.F. A rapid colorimetric method for the quantitative determination of the inorganic phosphorus in small amounts of serum. Jour. Biol. Chem. 50:329. 1922.
12. Yagoda, H. Radioactive Measurements with Nuclear Emulsions. John Wiley and Sons, Inc., New York. 1949.
13. Zilversmit, D.B., and A.K. Davis. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. Jour. Lab. Clin. Med. 35:155. 1950.
14. _____, and N.R. DiLuzio. Synthesis of phospholipids in diabetic dogs. Jour. Biol. Chem. 194:673. 1952.

IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 477-495

DEVELOPMENTAL MORPHOLOGY OF THE INFLORESCENCE AND FLOWER OF TRIFOLIUM PRATENSE L.[1]

Warren E. Picklum[2]

Red clover, Trifolium pratense L., has been the subject of numerous papers published during a period of more than a half century. Most of these studies have been concerned with agronomic and genetic problems and with insect relationships. Comparatively few of the papers have dealt with the developmental morphology of the plant. Similarly, most studies of the flower have been descriptive rather than developmental in approach.

An ontogenetic study of the red clover inflorescence in terms of the tunica-corpus concept of histogens is needed in order to understand the derivation of the flower and its organs and to determine the order of initiation of floral organs in the developmental sequence. A study of the ontogeny and structure of the nectary is of interest because of the close relationship between insect attraction and seed set in red clover. If the amount of nectar secreted by the flower is closely correlated with its attractiveness to bees, and if the amount of nectar produced is correlated with surface area or nectary volume, then, nectary size would be a valuable criterion in the selection of desirable plants.

This investigation was undertaken to determine the sequence of initiation and development of the floral organs, and to determine the time of initiation and extent of development of the nectary. A single variety, Emerson, was studied to provide a basis for subsequent morphological comparison of varieties and for interpretation of the results of experimental treatments.

REVIEW OF PERTINENT LITERATURE

Payer (14) was one of the first morphologists to realize the value of developmental morphology in the study of the flower in many plant families. Among the papilionaceous legumes, he studied Trifolium ochroleucum, Lathyrus sylvestris, and Lupinus varius. In T. ochroleucum, he found that the order of initiation of the floral whorls is calyx, corolla, first stamen whorl, second stamen whorl, and finally the pistil. Payer also noted that the order of appearance of the primordia in each whorl is "from front to rear". The floral organs appear first on the side of the flower abaxial to the inflorescence axis, and the other members of the same whorl arise progressively toward the adaxial side of the flower. He stated that the pistil arises "like a small leaf", and that the edges approach each other and fuse to form the ovary.

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[1] Contribution from the Botany and Plant Pathology Department, The Agronomy Department of the Iowa Agricultural Experiment Station, Ames, Iowa, and the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration of the U.S. Department of Agriculture. Project No. 1001. Journal Paper J-2318.

[2] The writer wishes to express his gratitude to Drs. J.E. Sass, C.P. Wilsie and E.A. Hollowell for their encouragement and guidance during this study.

Frank (8) described the early development of flowers of several legumes, including T. pratense, and found that the succession of initiation of the floral whorls is acropetal. In each of the legumes studied, he found that the carpel is initiated as a distinct unclosed ring around the axis of the flower.

Westgate (19) and his associates made observations on the development of the young flower of red clover, but they were primarily interested in the anthers, the maturation of the pollen and the development of the ovules. These authors considered the corolla tube to be a "staminal tube" to which the petals are attached, and stated that "Nector is secreted at the bases of the stamens and accumulates in the staminal tube around the base of the ovary". Martin (11) made a comparative study of the development of the ovule, embryo sac, and embryo in T. pratense, T. hybridum, T. repens, Medicago sativa, and Vicia americana, but he did not describe the development of the other floral members.

Grégoire (9) stated that the papilionaceous carpel arises from a closed annular primordium. This is in disagreement with the interpretation of Payer (14), who stated that the carpel arises from a leaf-like, crescent-shaped primordium, which later fuses at its edges. Bugnon (5) examined young flowers of Lathyrus vernus, T. pratense, and Lupinus perennis and found that the carpel arises in the manner described by Payer. Bugnon stated that Grégoire probably made his observations on older flowers in which the carpel edges had already fused.

These studies of the ontogeny of the papilionaceous flower did not consider the histogenesis of floral organs from the histogens of the shoot apex. The pioneer work of Hanstein (10), Schmidt (16), Schüpp (17), and numerous recent studies have indicated a need for the re-examination of the problems of organogeny in terms of the several histogen theories.

The secreting organs of flowers have been of interest to naturalists for centuries, and interesting theories, many purely teleological, have been propounded to explain the presence and functions of nectaries. Malphighi, Pontedera and Linnaeus were among the earlier botanists to note that bees were attracted by the sweetish juice produced by the glandular structures, but these observers believed that the nectar was produced for the nourishment of the young ovules.

Linnaeus' Nectaria florum, published in 1763, described floral secreting organs, which he designated as "nectaria", and their secretions as "nectar". By this time he was sure that bees and other insects, in their quest for nectar, transferred pollen to the stigmas of flowers and thereby insured pollination. Many writers disagreed with his classification of nectaries; others doubted that the nectaries were the source of the sweet liquid found in flowers.

Bravais (3) agreed with Linnaeus that the floral nectaries secrete the sweet liquid. He noted that the secretion of nectar usually started just before the shedding of pollen, and that the nectar was often visible after the pollen had disappeared from the anthers.

Much of the work up to this time attempted to clarify nomenclature and the complicated systems of nectary classification that had arisen since Linnaeus' work was published. Brongniart (4), one of the first to do creditable anatomical work on nectaries, determined the location and extent of "septal glands" in many genera of the Monocotyledoneae.

Martinet (12) studied the glandular hairs of plants; he considered these to be organs of secretion. He evolved an elaborate system for classifying glandular hairs, but he neglected entirely the more massive secreting structures found in flowers.

Behrens (1) was able to produce stained sections of nectaries and described the cell structure which is characteristic of nectary tissue. He also described and illustrated stomata in the epidermal layer of the nectary of a maple and several umbellifers.

Bonnier (2) presented and discussed thoroughly the historical background of research on the nature of the nectary. His work is devoted principally to the various aspects of insect attraction by flowers, and the physiology and anatomy of the nectary. In Vicia sativa he found that the nectary forms a ring at the base of the stamens which is most massive on the lower or keel side of the flower. He found stomata near the summit and on the inner side of this more prominent portion of the nectary. He described similar structural features in several other papilionaceous legumes that have diadelphous stamens.

Cammerloher (6) studied the attraction of insects to flowers by color, scent and nectar. He found that nectar is secreted through the stomata in the heavily cutinized epidermis of certain nectaries. He also found that nectar escapes through minute canals through the cuticle on the nectary of Euphorbia pulcherrima, and through small pores in the cuticle, and between the epidermal cells of the nectary of Grevillea preissii.

Ewert (7) made a study of the physiology of nectaries and hydathodes; he also made some anatomical observations. Sperlich (18) illustrated nectar secretion by plasmoptisis of the epidermal nectary cells of Ranunculus acer. Moore (13) has assembled from the literature evidence that the secretory disc in the Phaseoleae is probably of staminal derivation.

MATERIALS AND METHODS

Plant material for this study of red clover was obtained from plots of the Emerson variety at the Agronomy Farm of the Iowa State College Experiment Station at Ames, Iowa. The 1948 collections were made from an established stand. The 1949 and 1950 collections were made from plots planted in the spring of 1949. Stem tips were collected at intervals during May of 1948 and during April and May of 1949. The 1950 collections were made before and after the spring thaw.

Dissections were made to determine the condition of the post-dormant apex and to verify the date of initiation of the inflorescence. Vegetative apices and very young inflorescences were killed whole after removal of the mass of investing leaf bases. Larger inflorescences were subdivided by cutting them into segments, each bearing several flowers. Flowers that were large enough to be handled separately were cut from the inflorescence axis and killed whole.

Killing solutions of the chrome-acetic-formalin type, principally Craf III, were used for both vegetative and flowering apices as well as for older flowers. The dioxan-normal butyl alcohol dehydration series was used in the paraffin process (15). All material was sectioned ten microns in thickness.

Hemalum, iron hematoxylin, and safranin-fast green staining combin-

ations were used; the latter combination was by far the most satisfactory.

Each young inflorescence, being a compact raceme of flowers, provided sections of several dozen flowers of slightly different ages when sectioned serially. Because of the angle at which each flower is attached to the inflorescence axis, it was necessary to section some of the younger heads at an angle oblique to the axis in order to obtain transverse and longitudinal sections of individual flowers.

OBSERVATIONS

Growth and Flowering Habit of the Plant

The stem apex of the seedling plant of red clover produces the foliage leaves during the early growth of the plant. A bud is produced in the axil of each leaf, and, as the lower leaves die, the bud grows rapidly and becomes an axillary branch. The rapid growth and assumption of dominance by these lower axillary branches produce the characteristic bushy, many-stemmed, thick-crowned plant, on which the primary axis is not readily distinguishable.

As growth begins in the spring, the larger of the axillary branches begin to elongate. Initiation and early development of the inflorescence takes place during this period. Elongation of the branch is progressive during the early growth of the terminal inflorescence, but slows or stops during the period of maturation of the head.

The bud in the axil of each leaf on the flowering stem is capable of producing an inflorescence. The terminal inflorescence of a main flowering stem is the first to be initiated and to mature. The other inflorescences produced by secondary branches on a flowering stem mature in essentially basipetal order.

A secondary rank of branches is produced in the leaf axils below the terminal inflorescence of each main flowering stem. These secondary branches may have from one to six internodes; those with the larger number of internodes are found lower on a main flowering stem.

A tertiary rank of branches may be produced in the axils of leaves on these secondary branches, and the tertiary branches usually have one or two internodes.

The growth and flowering pattern of Emerson red clover may be summarized as the production of succeeding ranks of axillary branches, each of which produces a terminal inflorescence. The number of succeeding ranks produced on a flowering stem seems to be dependent upon the vigor of the plant, length of the growing period, and competition with developing seeds on the older ranks of branches.

Seasonal Initiation of the Inflorescence

Collections made in May, 1948, showed that the inflorescence had already been initiated. In the spring of 1949, collections were made from the above plants at intervals, beginning on April 7th, at which time all apices were definitely vegetative.

On April 26th of 1949 and April 27th of 1950, flower[1] primordia could be seen at the bases of the more advanced apices under a dissecting microscope. The appearance of these first flower primordia provides visible proof that transition from the vegetative to the flowering phase has taken place, and provides the approximate date of floral initiation.

Prior to the above date, flower primordia are not distinguishable, but enlargement and a change of shape of the apex is evident. This change of shape, which characteristically precedes the appearance of flower primordia, consists of a lateral extension and elongation of the head toward the axil of the last-produced leaf; whereby the head becomes somewhat flattened (Figs. 2-5). This change, which is detectable in some apices as early as April 13th, is the earliest morphological evidence of the transition from the vegetative to the flowering phase.

Collections were made at intervals extending well into cold weather during the autumn of 1949, to determine whether some initiation of inflorescences takes place in the fall. A few inflorescences are apparently initiated late enough in the season to be present at the onset of freezing weather. When such inflorescences are thawed out, they are found to be disorganized and necrotic. In contrast, the vegetative apex is not damaged by the same low temperatures and remains a translucent, glistening dome, with no evidence of disorganization. The presence of normal, undamaged, vegetative apices on axillary buds within a millimeter or two of frozen, disorganized apices, which had visibly undergone some floral differentiation, indicates that, after the change to the flowering phase occurs, apices of red clover cannot withstand winter conditions.

Several collections were made early in 1950, before and after thawing of the ground, to determine the incidence of winter-killing of stem apices. The apices collected before thawing appeared to be in good condition, as judged by their appearance after dissection. Nearly all apices appeared to be in good condition after thawing normally in the field, but a few were collapsed or disorganized. The surviving stem apices were all in the vegetative condition. It is evident that inflorescence primordia do not overwinter and that, on established plants, all flowers of a given season are initiated during the early spring of that season.

Initiation of Inflorescence and Flower Primordia

The dome-shaped vegetative stem apex has a two-layered tunica (Fig. 1). Cell divisions in the outer tunica layer are exclusively anticlinal, whereas, in the second tunica layer, occasional periclinal divisions occur at the sides of the dome. The corpus is composed of cells with irregular orientation, in which cell division occurs in random planes. No specific corpus initials are recognizable.

Leaf primordia are produced near the top of the stem tip by activation of cells of the inner layer of the tunica and of one or two adjacent layers of the corpus. The first evidence of leaf initiation consists of enlargement of cells in this zone, followed by periclinal divisions. Continuity

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[1] The term floret has been used extensively in the literature for the small flowers of certain compact inflorescences. The term should probably be restricted to the specialized flower of the grass together with its enclosing lemma and palea. Therefore, the term flower will be used in this paper to refer to the individual member of the red clover inflorescence.

and identity of the tunica with the outer cell layers of the developing organ are maintained by anticlinal divisions in the tunica. The leaf primordia are produced alternately, and the three lobes of the leaflets are evident by the time the leaf primordium exceeds the height of the stem apex.

The stimulus that brings about the morphological initiation of the inflorescence and flowers is known as induction. The first morphological evidence that induction had taken place is the rapid increase in the width of the stem apex from an average of .132 mm. to approximately .360 mm. No foliage leaves are initiated after this time.

This rapid increase in size is produced by increased meristematic activity in a zone of four to six cell layers on the periphery of the apex. These layers stain deeply, and many mitotic figures are evident. The two tunica layers remain distinct, but periclinal and oblique divisions in the second tunica layer become more frequent. The delineation of this broad zone of meristematic activity is the first histological evidence of the initiation of the flowering phase (Fig. 2).

Accelerated activity at the sides of the apex, in the plane of leaf initiation, causes the inflorescence axis to become somewhat flattened. The lateral extension of the axis is slightly more pronounced over the axil of the last-produced leaf (Figs. 2-5). At this stage, the first flower primordium becomes evident (Fig. 5).

The flower primordium is initiated by enlargement of cells of the second tunica layer and the cells of the underlying active zone of the corpus. The initiation of flower primordia on the inflorescence axis is therefore similar to leaf initiation. Anticlinal divisions in the outer tunica layer accompany this protrusion, and the first and second tunica layers of the flower primordium retain their identity with those of the inflorescence axis. Activity of the outer corpus layers of the new flower primordium, accompanied by anticlinal divisions in its two tunica layers, produces a nearly hemispherical protuberance on the surface of the inflorescence axis.

The first flower primordium appears in the axil of the next-to-last-produced leaf (Fig. 5). From this point, the initiation of flower primordia is progressively acropetal and lateral. The youngest primordia appear in the region above the last-produced leaf, distal to the first-formed primordium (Figs. 3-5).

The upward meristematic extension of the inflorescence axis, and subsequent increase in the number of flowers, seems to be correlated with the vigor of the flowering stem, and may be one of the main factors determining the number of flowers per head.

Organogeny of the Flower

As the hemispherical flower primordium begins to enlarge, a ridge is initiated and progressively encircles the base of the primordium (Fig. 6). This ridge enlarges very little and remains at the base of the flower as a rudimentary bract. The flower primordium in the axil of this bract becomes nearly spherical, and then becomes somewhat flattened by lateral meristematic activity. The cells of the second tunica layer enlarge anticlinally, and some periclinal divisions occur prior to initiation of the floral appendages.

The first floral member to appear is the abaxial sepal primordium, which arises as a small protuberance on the lower side of the flower primordium (Fig. 6). A sepal primordium arises next on either side of the abaxial one, and the two adaxial sepal primordia appear immediately afterward, completing the first whorl of floral appendages. This progression of initiation of the five members of a whorl from the abaxial to the adaxial side of the flower occurs during formation of each whorl of floral members. The abaxial sepal grows rapidly and remains dominant in size throughout the life of the flower.

The primordia of the five petals arise in a whorl slightly centripetal to the sepal whorl, and alternate with the sepals. The keel petal primordia appear first, followed by the wing and standard petal primordia.

The five primordia of the first stamen whorl are alternate with the petals and appear simultaneously with the petal primordia. These two whorls appear at first to be members of the same whorl, but the widening of the flower and the lateral extension of the petal primordia show that the stamen whorl is distinct and centripetal to the petal whorl.

The second whorl of five stamens arises slightly later, centripetal to the first whorl and alternate with the first stamens. Each primordium of the second stamen whorl is opposite a petal primordium and appears to be adnate at its base to the petal primordium (Fig. 7).

Evidence of the initiation of the pistil can be seen as soon as primordia of the second stamen whorl becomes visible. The initiation of the pistil begins with acceleration of meristematic activity at a point just within the inner stamen whorl, on the abaxial side of the flower apex. This activity extends laterally from this point around the flower axis and produces a primordium that is U-shaped in cross-section. In longitudinal section the primordium is conical because of the earlier initiation and more rapid growth of the abaxial side (Fig. 7). The pistil elongates rapidly until it is twice the height of the stamens. The greater elongation of cells in the dorsal region causes the pistil to curve toward the axis of the flower (Figs. 7-8). Continued cell division throughout this tissue, and continued meristematic activity at the margins, followed by ontogenetic fusion of the margins, produce the closed monocarpellate pistil with a hollow style. The slightly recurved, papillose stigma is derived from tissue of the abaxial side of the style apex.

The two ovule primordia are produced inward from the fused carpel margins immediately after fusion of the margins. The ovules are produced at nearly the same level, one on each of the marginal placentae. Subsequent enlargement of the ovules causes displacement from the nearly side-by-side position, so that at the time of fertilization, one ovule is essentially above the other. The development of the ovule, embryo sac, and seed has been described in the literature.

Development of the flower parts after their initiation consists of accelerated meristematic addition of cells, rapid cell enlargement, and differentiation. Meristematic activity, subjacent to and within the tissue between the bases of the sepal primordia, produces a continuous calyx tube. The free portions of the sepals enlarge by apical and marginal meristematic activity. The calyx tube grows rapidly, reaches its mature proportions far in advance of the other floral organs, and encloses the developing flower until the final elongation of the corolla tube begins.

The distal portions of the stamens of the outer whorl expand to produce the anther before lateral development brings the petal bases into close proximity (Fig. 8). The anthers of the outer whorl are elevated by their filaments above the developing anthers of the second stamen whorl.

Meristematic activity of the tissue at the base of nine of the stamens elevates this region of the receptacle, and the bases of the stamens are thus merged into a common tissue which forms a semi-circular tube (Fig. 9). The tenth stamen, which is adaxial and adjacent to the standard petal, remains free and is attached to the receptacle (Fig. 9).

By lateral meristematic extension, the bases of the petal primordia become contiguous. Subsequent activity in the underlying region elevates the petals and forms a continuous ring of meristematic tissue. Elongation of the cells of this tissue produces the corolla tube. The zone of elongation is above the point of attachment of the free stamen, and this stamen is not elevated on the tube.

Microspores are evident in the anthers before appreciable elongation of the corolla tube takes place. Elongation of the style and the filament of the free stamen keeps pace with the elongation of the tube. Expansion of the alar processes during the elongation serves to hold the style and free stamen in position within the semi-circular stamen tube. Dehiscence of the anthers takes place before elongation of the tube is complete and before the petals have reached their full size. Pollen fills the space between the fused keel petals and is prevented from falling down the tube by the alar processes.

Development of the Nectary

The mature nectary of red clover is an irregularly lobed ring of secretory tissue situated on the receptacle at the base of the corolla tube (Fig. 13). The portion of greatest bulk is found on the abaxial side of the flower; comparatively little nectary tissue is present near the base of the free stamen on the adaxial side of the flower.

The nectary may first be recognized at the stage of flower development when pollen quartets or microspores are first distinguishable. At this time, the bases of the petals and stamens have become confluent by elevation of the underlying tissue. Early evidence of nectary development is the presence of immature stomata in the epidermis of the receptacle on the abaxial side of the flower (Figs. 14-15). The immature guard cells are recognized by their shape and staining properties; they are rounded in outline, stain more deeply than the other epidermal cells and are separated by obviously thick cell walls (Fig. 14). Subsequent development consists of some enlargement, and separation of the inner walls thereby forming the open pore (Fig. 16). No sub-stomatal chamber is present.

The tissue underlying these stomata is distinguishable from the tissue of the corolla and receptacle by the more dense cytoplasm, small vacuole and the incidence of cell division in random planes. After initiation of the nectary, rapid cell division produces the raised, irregularly lobed ring of tissue characteristic of the mature nectary (Fig. 13). The lobes though irregular in size and shape, are found to be generally alternate with the vascular bundles of the stamens.

The secretory tissue is composed of a homogeneous mass of small cells containing very dense cytoplasm with small vacuoles (Figs. 13, 16). The epidermis of the nectary is similar to and continuous with that of the inside of the corolla tube and the outside of the ovary. Further development of the nectary includes continued cell division and enlargement of the nectariferous tissue during the final enlargement of the flower.

DISCUSSION

The observed development of successive branch orders of Trifolium pratense L. shows that the number of inflorescences is dependent upon the longevity and vigor of the main flowering branch. A spindly stem produces a terminal inflorescence, but its secondary branches are weak and their terminal inflorescences may never develop. A similar relationship is evident with regard to the number of flowers in an inflorescence. The inflorescence on especially vigorous stems is elongate and bears perhaps twice the number of flowers found in the inflorescence on a spindly stem. The inflorescence on an especially weak stem is often only a fringe of mature flowers. This is caused by abortion of the flowers on the upper portion of the inflorescence axis, probably because of competition with the more advanced flowers.

This comparative flower-producing capacity of different apices is evident microscopically during the initiation of flowers. Few flower primordia are produced on the floral apex of a weak stem. The apex of a more vigorous stem continues to elongate by the meristematic addition of tissue; further flower initiation occurs, and an elongated inflorescence with many more flowers is produced.

Inasmuch as flower initiation takes place within a relatively short time after the beginning of the flowering phase, the size and vigor of the flowering stems are particularly important in determining the number of flowers produced per head. The general vigor of the plant during the first season's growth determines the size and potential vigor of the plant during the following season. Therefore, the more vigorous plants may be expected to produce more vigorous stems, more inflorescences, and more flowers per inflorescence.

This pattern of growth and flower initiation applies to plants that had become established the season before the first seed crop is expected. Red clover is ordinarily planted in the spring with a cover crop; the seedlings become established, but relatively few plants in a stand become advanced enough to flower the first year. However, some seedlings, under favorable conditions, develop into vigorous plants which flower late in the season. The initiation of flowering in these plants probably depends upon a minimum level of vegetative development. Such plants were not studied because they are of little importance in seed production.

The cyto-histology of vegetative and floral organogeny in red clover can be interpreted best in terms of the tunica-corpus concept. The tunica and corpus of the vegetative apex retain their identity during the transition to the flowering phase. The volume of the corpus is increased by the addition of cells on its periphery by activity of the outer corpus layers and by enlargement of the inner cells of the corpus. Expansion of the surface area is made by anticlinal divisions in the tunica layers. The persistence

of the meristematic activity which continues the elongation of the distal end of the floral apex, is not different from the purely vegetative growth which elongates the stem.

The identity of the tunica layers is maintained during the formation of the flower primordia; however, the second tunica layer undergoes some enlargement in an anticlinal direction, immediately before initiation of the floral members. The primordia of floral members are initiated in the same manner as leaf primordia. These observations do not support Grégoire's contention that the vegetative apex and the floral apex are distinctively different entities having different patterns of growth.

The acropetal order of initiation of the floral whorls agrees with the findings of other workers on species of Trifolium, Lotus and Soja, and may be true throughout the Leguminosae. The order of appearance of the primordia of each floral whorl from the abaxial toward the adaxial side of the flower agrees with the findings of Payer (14) and Frank (8). The initiation and direction of differentiation of the nectary proceeds adaxially in the same manner as in the floral whorls.

The red clover nectary is histologically similar to the nectaries of many other plants described by other investigators. Highly irregular lobing of the nectary occurs in many species. This irregularity does not facilitate accurate measurements of the volume or surface area of the nectary in the variety of red clover in this study. One of the objectives of the present study was to explore the possibility of using structural features of the nectary in varietal comparisons. The variability of structural features of the nectary of Emerson indicate that surface or volume measurements would not be suitable as criteria for comparison of varieties of red clover.

SUMMARY

A study of the Emerson variety of red clover, Trifolium pratense L. was made to determine, a) the approximate date of floral initiation, b) the histological transition of the stem apex from the vegetative to the flowering phase, c) the organogeny of the flower, and d) the development of the nectary.

Each inflorescence is terminal on one of the successive ranks of branches.

On plants grown at Ames, Iowa, the most advanced apices had floral primordia on April 26th in 1949 and April 27th in 1950. Some indications of transition were detectable as early as April 13th.

Inflorescence primordia that are initiated in the fall do not overwinter; only vegetative apices survive; therefore, flowers that attain anthesis are initiated during the current growing season.

The dome shaped vegetative stem apex has a two-layered tunica. Transition to the flowering phase is characterized by cessation of leaf initiation, rapid increase in size, and flattening of the apex by lateral extension in one plane.

The first flower primordia appear at the base of the axis on the side opposite the last-produced leaf, and successive floral primordia arise acropetally. A flower primordium is a hemispherical protuberance subtended by a rudimentary bract.

The whorls of floral organs appear in the following acropetal order: sepals, petals, 1st stamen whorl, 2nd stamen whorl, and the pistil. Members of a whorl appear first on the abaxial side of the apex; initiation of members then proceeds toward the adaxial side.

Members of the calyx whorl are initiated as separate primordia, which become elevated on a tube formed by meristematic activity of a basal zone.

Petal primordia develop into the free members of the corolla. The keel is formed by fusion of the edges of two petals.

Stamen primordia arise as separate structures. Nine of the stamens become elevated on the corolla tube by the activity of a basal annular meristem, whereas the adaxial stamen remains free.

The carpel arises as a meristematic crescent which elongates and undergoes closure and fusion of the edges.

Initiation of the nectary becomes evident when the guard cells of the stomata of the nectary begin to differentiate. At this stage, pollen quartets are present in the anthers.

The mature nectary is an irregularly lobed ring of homogeneous tissue at the base of the corolla tube. It is not known whether the stomata of the nectary are in any way functional.

The irregular lobing, the variation in depth within the receptacle, and the indistinct inner boundaries of the secretory tissue preclude accurate measurement of the nectary of the red clover flower. Superficial measurements or estimates of volume or surface for varietal comparisons, therefore, would have little validity.

REFERENCES

1. Behrens, W.J. Die Nectarien der Blüthen. Flora. 62:60-64. 1879.
2. Bonnier, G. Les Nectaires. Paris, Masson. 1879.
3. Bravais, L. Examen organographique des nectaires. Ann. Sci. Nat. ser. 2. 18:152-184. 1842.
4. Brongniart, A. Mémoire sur les glandes nectarifères de l'ovaire. Ann. Sci. Nat. ser. 4. 2:5-23. 1854.
5. Bugnon, P. Organogénèse et déhiscence de la gousse des papilionacées. Bull. Soc. Bot. France. ser. 5. 72:445-448. 1925.
6. Cammerloher, H. Blütenbiologie, I. Wechselbeziehungen zwischen Blumen und Insekten. Berlin, Gebrüder Borntraeger. 1931.
7. Ewert, R. Die Nektarien in ihrer Bedeutung für Bienenzucht und Landwirtschaft. Leipzig, Leipziger Bienen-Zeitung. 1932.
8. Frank, A.D. Über die Entwicklung einiger Blüthen mit besonderer Berücksichtigung der Theorie der Interponierung. X. Die Papilionaceen. Jahrb. Wiss. Bot. 10:204-243. 1876.
9. Grégoire, V. L'organogénèse de l'ovaire et la déhiscence du fruit (Note préliminaire). Bull. Soc. Roy. Bot. Belgique. 56:134-140. 1924.
10. Hanstein, J. Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen. Festschr. Niederrhein. Gesell. Natur-und Heilkunde. pp. 109-143. 1868.
11. Martin, J.N. Comparative morphology of some Leguminosae. Bot. Gaz. 58:154-167. 1914.

12. Martinet, M.J. Organes de sécrétion des végétaux. Ann. Sci. Nat. Bot. ser. 5. 14:91-232. 1872.
13. Moore, J.M. The vascular anatomy of the flower in the papilionaceous Leguminosae II. Amer. Jour. Bot. 23:349-355. 1936.
14. Payer, J. B. Traité d'organogénie comparée de la fleur. Paris, Masson. 1857.
15. Sass, J.E. Botanical microtechnique. Sec. ed. The Iowa State College Press. 1951.
16. Schmidt, A. Histologische Studien an phanerogamen Vegetationspunkten. Bot. Arch. 8:345-404. 1924.
17. Schüpp, O. Meristeme. Handb. der Pflanzenanat. Abt. 1, T. 2, Bd. IV. 1926.
18. Sperlich, A. Das trophische Parenchym. B. Exkretionsgewebe. Handb. der Pflanzenanat. Abt. 1, T. 2, Bd. IV. 1939.
19. Westgate, J.M., et al. Red clover seed production. USDA Bull. 289. 31 pp. 1915.

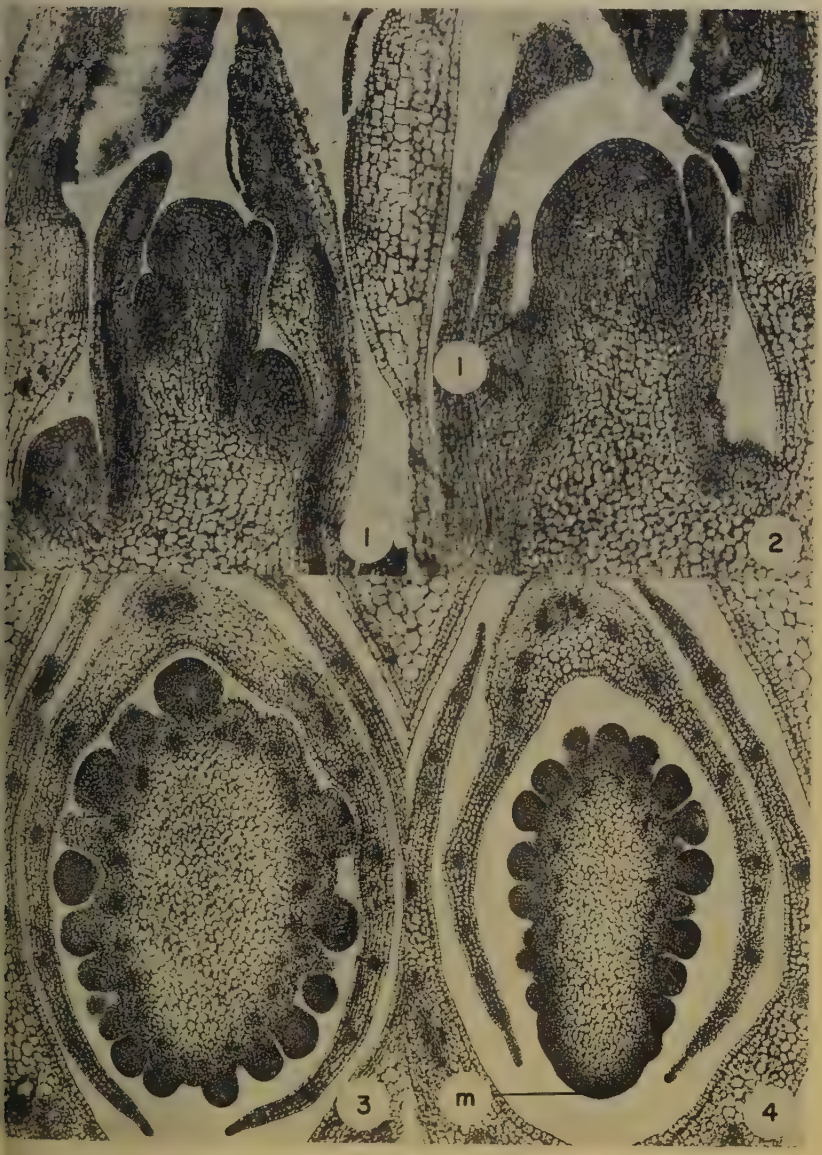
PLATE I

Fig. 1. Vegetative apex showing foliage leaf primordia. 115x.

Fig. 2. Stem apex in transition from the vegetative to the flowering phase, showing characteristic lateral expansion. Last-produced leaf (1). 115x.

Fig. 3. Transverse section showing floral primordia of different ages a the same level on the flowering apex. 93x.

Fig. 4. Section of same apex at higher level showing zone of continue meristematic activity (m). 93x.


1
1
2
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m
4

PLATE II

Fig. 5. Stem apex with first flower primordia (pr). Last-produced leaf (1). 93x.

Fig. 6. Flower primordium showing rudimentary bract (br) and abaxial sepal primordium (s). 400x.

Fig. 7. Young flower showing members of five floral whorls: calyx (c), petal (pe), first stamen whorl (st_1), second stamen whorl (st_2), pistil (p). 230x.

Fig. 8. Older flower showing expansion of anther of first stamen whorl and enclosure of floral parts within calyx tube before petal growth is accelerated. 115x.

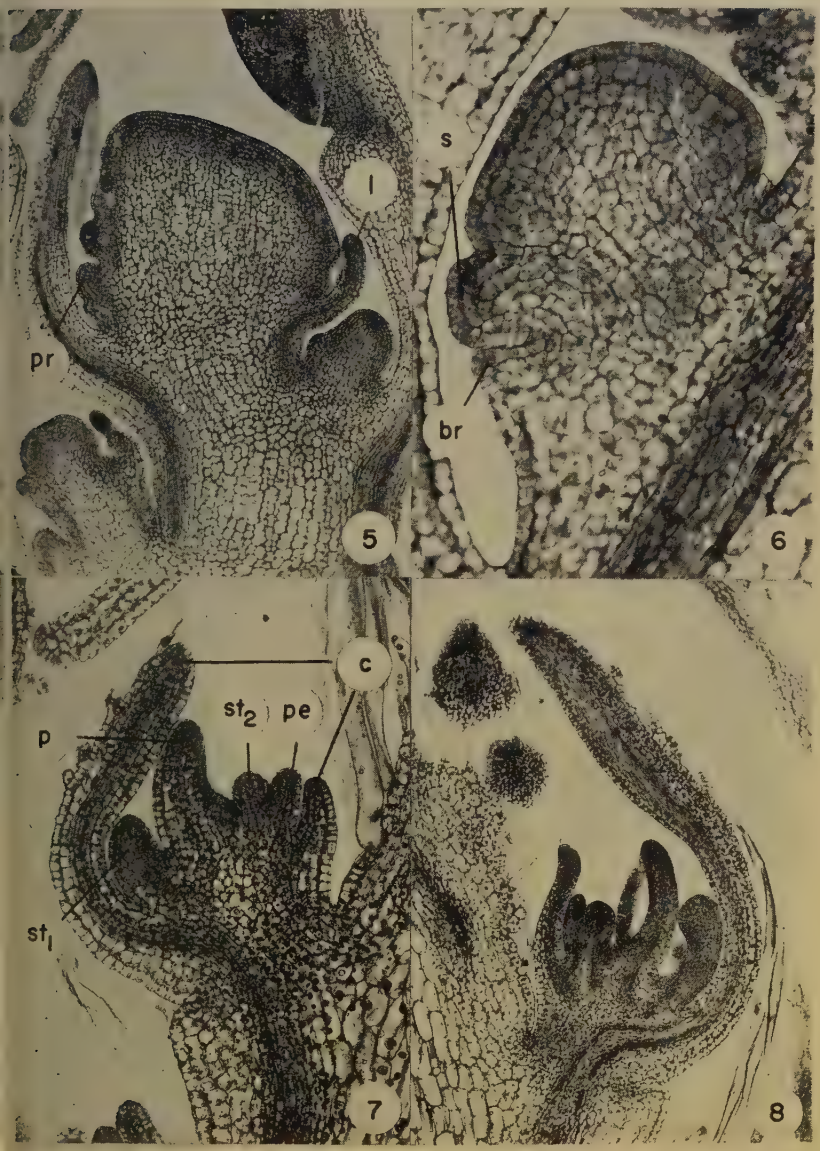
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PLATE III

Figs. 9 - 12. Transverse section of older flower at four levels to show stamen tube (t) and merging of floral members with the corolla tube. 115x.

Fig. 9. Floral members separate above point of attachment. Nine stamen filaments are coalesced at their bases.

Fig. 10. Section 30 microns below Fig. 9.

Fig. 11. Section 70 microns below Fig. 10. Note that coalescence of flower parts into a common tube does not take place at the same level.

Fig. 12. Section 60 microns below Fig. 11. All petals and stamens united to a common tube immediately above the receptacle, before elongation of the corolla tube.

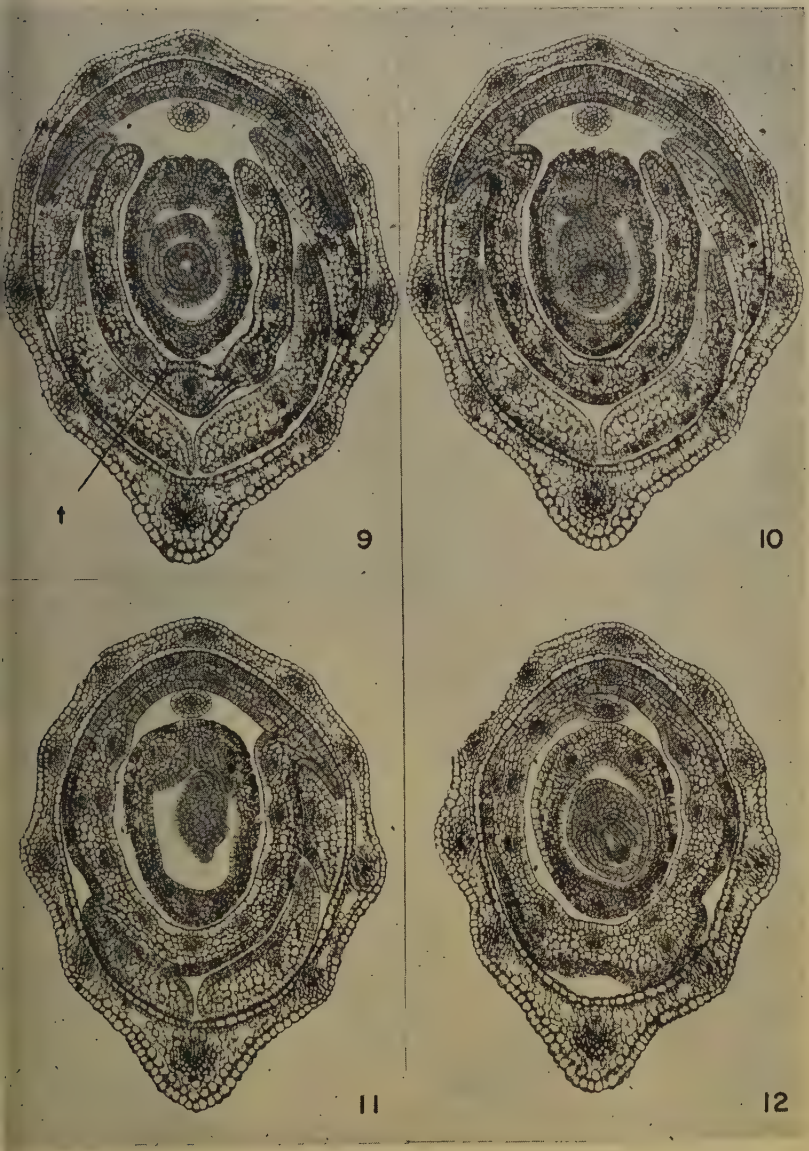
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PLATE IV

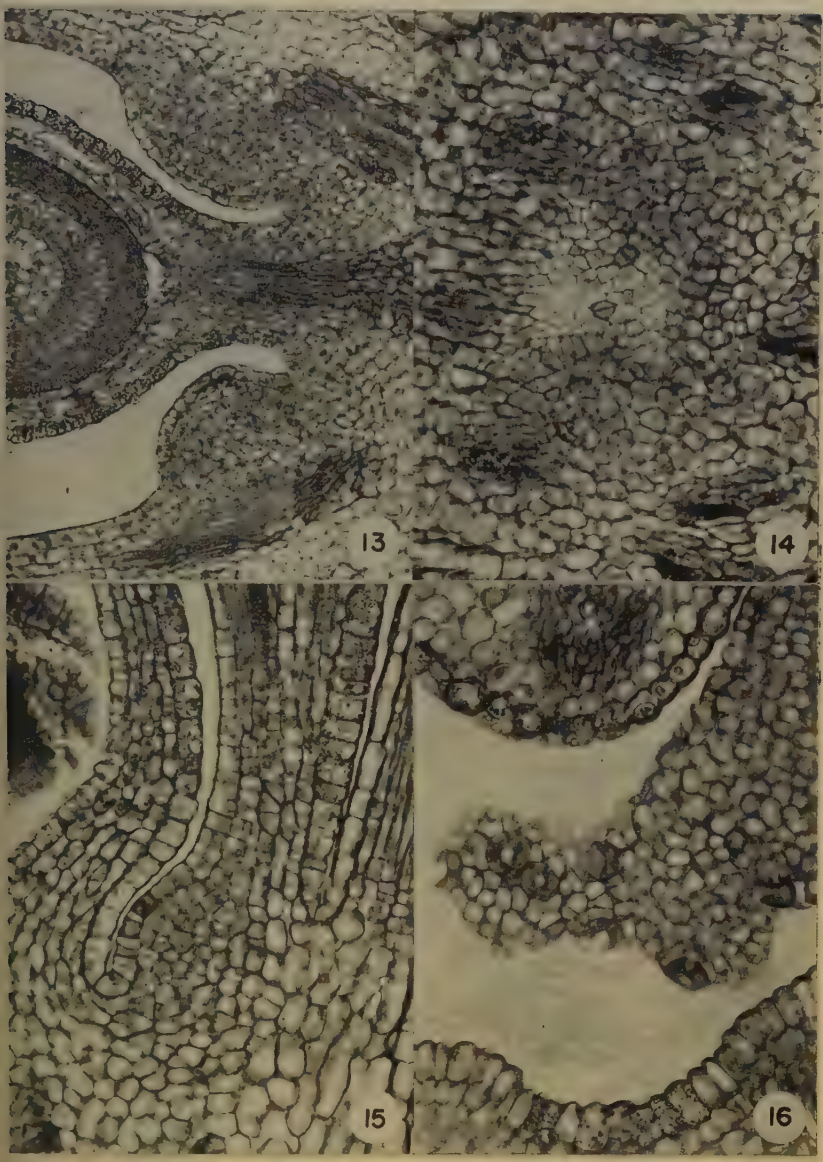
Figs. 13 - 16. Nectary formation.

Fig. 13. Longisection of nectary showing homogeneous mass of secretory tissue and stomata in the epidermal layer. 230x.

Fig. 14. Oblique section of young flower showing stomate initials (center) on surface of earliest recognizable stage of nectary formation. 400x.

Fig. 15. Longitudinal section of nectary soon after initiation, showing early stage of development of secretory tissue. Note stomate initial. 400x.

Fig. 16. Transverse section of nectary showing stomata in epiderma layer. 400x.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IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 497-502

LIPID PHOSPHORUS IN CALF BLOOD PLASMA*

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A review of the literature indicates that a variety of analytical procedures has been employed in the estimation of blood plasma phospholipids. Moreover, it becomes apparent that comparison between various reported values is not justified because of fundamental differences in the techniques employed. Briefly, the separation of blood plasma phosphatides (mainly lecithin, cephalin and sphingomyelin) generally has been accomplished either by extraction with nonaqueous solvents or by precipitation with trichloroacetic acid. Colorimetric, titrametric, or microgravimetric procedures subsequently were employed for the estimation of the "lipid" phosphorus.

The phospholipid values previously reported (13) for calf blood plasma included only those phosphatides which were insoluble in acetone and $MgCl_2$, but it was recognized that other "lipid" phosphorus components may have been present. In an effort to achieve a more complete understanding of the nature of the lipid phosphorus in calf blood plasma, it seemed desirable to measure the plasma phosphatides by several extraction and precipitation techniques.

EXPERIMENTAL

Four Ayrshire calves from the Iowa State College dairy herd were allowed to remain with their respective dams for 3 days following birth. Subsequently they were fed whole milk, medium quality alfalfa hay, and a simple concentrate mixture.

Samples of venous blood (heparin anticoagulant) were drawn at approximately 6 days of age and at weekly intervals thereafter for 6 weeks. After centrifugation, the plasma was subjected to the analytical treatments shown in the flow diagram (Fig. 1). An aliquot of plasma was treated with trichloroacetic acid to precipitate protein and phospholipids according to the procedure of Zilversmit and Davis (14). From another aliquot of plasma, the lipids were extracted with alcohol-ether (3:1), as described by Zaletel et al. (13), and a portion of the extract was reserved for phosphorus analysis. The remainder of the extract was evaporated under reduced pressure to remove the solvent. The residue was extracted with Skelly A (b.p. 32-36° C.) and the remaining portion was dissolved in water. The Skelly A extract and the aqueous phase were combined and agitated to assure removal of all suspended material in the petroleum ether extract. After separation of the liquid phases, the petroleum ether

* Journal Paper No. J-2342 of the Iowa Agricultural Experiment Station, Ames. Project No. 814.

layer was transferred to a volumetric flask. Three subsequent extractions of the aqueous phase were made with redistilled Skelly A and the extracts were combined with the original Skelly A extract. An aliquot of the combined Skelly A extract was reserved for phosphorus analysis and another aliquot taken for the precipitation of phospholipids with acetone and $MgCl_2$ by the method recommended by Bloor (2), Boyd (3) and others (8, 11). The acetone-insoluble phosphatides subsequently were dissolved in moist ethyl ether.

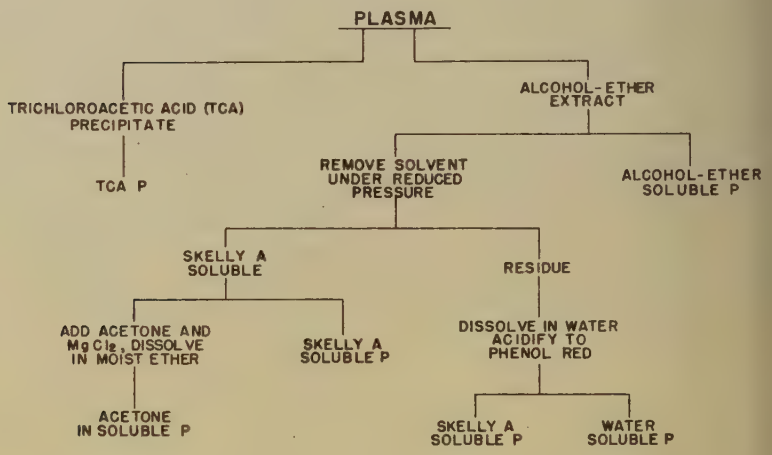

Fig. 1. Flow diagram for the separation of plasma "lipid" phosphorus fractions.

The residual water-soluble portion was acidified to phenol red with 25 per cent sulfuric acid and subsequently was extracted with Skelly A (residue-Skelly A fraction). The aqueous portion contained the residue water-soluble phosphorus.

All of the fractions prepared above were analysed for "lipid" phosphorus by a modification of the procedure of Zilversmit and Davis (14) which involved digestion with perchloric acid and a few drops of nitric acid and subsequent colorimetric measurement of phosphorus.

The Allen volumetric method (1) was employed to measure the plasma fat value for each sample to permit a comparison with trends in the various "lipid" phosphorus fractions.

RESULTS

The results of the extraction and precipitation methods for estimating the various "lipid" phosphorus values in calf blood plasma are presented in Fig. 2. The alcohol-ether extractable phosphorus values compared favorably with those obtained by trichloroacetic acid precipitation during most of the experimental period. Throughout the experiment the Skelly A-soluble and the acetone-insoluble phosphorus fractions were essentially parallel, the former being consistently higher. The residue Skelly A-soluble and residue water-soluble fractions contained significant quantities of phosphorus which accounts, at least in part, for the observed differences between the Skelly A and the alcohol-ether fractions. Allen fat values followed quite closely the trends exhibited by the alcohol-ether and the trichloroacetic acid fractions.

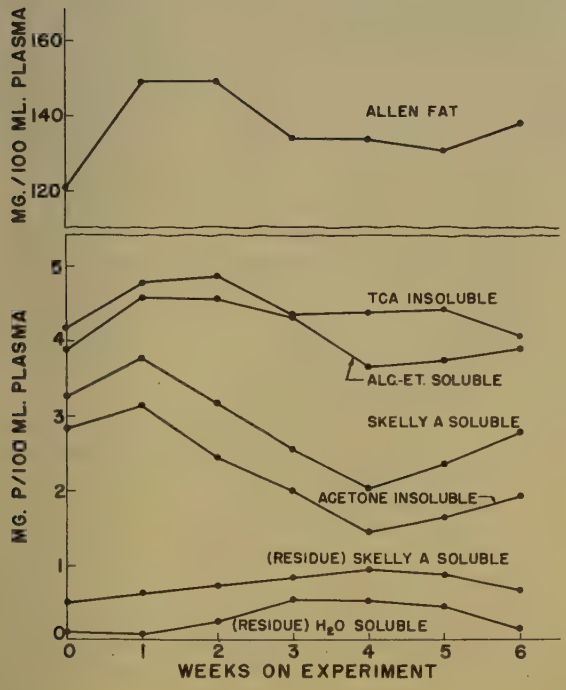

Fig. 2. Changes in the various plasma "lipid" phosphorus values in young dairy calves.

In general, the solvent-extracted and the trichloroacetic acid-precipitable phosphorus values reached maxima at 1 week, minima at 4 weeks, and tended to increase through the remainder of the experimental period Table 1 summarizes the mean "lipd" phosphorus and Allen fat values for the 1-, 4-, and 6-week periods.

TABLE 1

Plasma "Lipid" Phosphorus and Allen Fat Values in Young Dairy Calves

Weeks on experiment	"Lipid" Phosphorus						
	TCA precipitable	Alcohol-ether soluble	Skelly A soluble	Acetone insoluble	Residue: Skelly A soluble	Residue: H_2O soluble	Allen fat
	(mg./100 ml.)						
1	4.78* ±0.31	4.59 ±0.31	3.75 ±0.39	3.14 ±0.51	0.63 ±0.23	0.09 ±0.01	149.3 ± 13.1
4	4.38 ±0.39	3.65 ±0.64	2.03 ±0.53	1.43 ±0.48	0.92 ±0.31	0.53 ±0.36	134.3 ± 14.4
6	4.06 ±0.25	3.91 ±0.29	2.79 ±.12	1.91 ±0.28	0.69 ±0.14	0.16 ±0.03	138.9 ± 13.9

*Mean value ± standard error

DISCUSSION

Stewart and Hendry (11) reported that the phosphorus in alcohol-ether extracts of plasma may be used as a measure of the total "lipoid" phosphorus. More recently, Zilversmit and Davis (14), working with human and dog blood plasma, found that phosphorus values obtained from alcohol ether extracts compare favorably with phosphorus values obtained by the trichloroacetic acid precipitation technique. The present study with young dairy calves is essentially in agreement with the findings of the latter workers. However, fractionation of the alcohol-ether soluble phosphorus shows that several phosphorus components are involved.

The need for thorough washing of the Skelly A extracts with water prior to the precipitation of true phosphatides with acetone and $MgCl_2$ has been emphasized by Wittcoff (12). Moreover, Folch and Van Slyk (5) have shown that considerable quantities of phosphorus (3-15 per cent passed into the water washings. Further evidence of contaminants in petroleum ether extracts has been reported by Christensen (4) and Sinclair (9). In the present study the phosphorus removed by washing was separated, following acidification, into a Skelly A-soluble fraction and a water soluble fraction. The nature of the phosphorus-containing components in these fractions is not clear, but there is some evidence (9, 10, 12) that the action of heat and of plasma lecithinases (which may be partially soluble in alcohol-ether) may result in some hydrolysis of the phosphatides

The extent to which such reactions may occur during fractionation has not been ascertained, but the removal of alcohol-ether and subsequent extraction with Skelly A possibly may have altered the physical and solubility characteristics of the phosphatides.

Most of the phosphorus present in the Skelly A extract was precipitated with acetone and $MgCl_2$ and subsequently was soluble in moist ether. Kirk, Page and Van Slyke (7) observed that some of the precipitated phospholipid from organ tissues and blood did not dissolve in moist ether. This fraction was shown to be diaminophosphatide primarily, and it was recognized that other phosphatides were present in the ether-insoluble material. Sinclair and Dolan (8) demonstrated that this ether-insoluble fraction was not entirely sphingomyelin and further that it represented a portion of all the phosphatides present. These workers also found that the amount of ether-insoluble phosphatide was dependent largely on the source of material and on the concentration of $MgCl_2$ employed in the precipitation of the phosphatides.

It has been demonstrated (6) that the type of dietary lipid fed to young calves does not alter appreciably the relative proportions of the various plasma lipids. This relationship is confirmed, at least in part, by the observation that similarity in trends occur between the Allen fat values (which appear to include essentially all the plasma lipids except phospholipids and "free" fatty acids (13)) and the phosphorus in the alcohol-ether and trichloroacetic acid fractions.

Changes in the various "lipid" phosphorus values during this experiment possibly may be attributable to normal changes in the dietary of the animals. Subsequent to 3 weeks on experiment the milk intake was gradually reduced; concomitantly the consumption of concentrate mixture and of hay was gradually increased. Another factor which may be involved is the progressive development of rumen function.

Although this investigation demonstrates that several types of lipid phosphorus-containing compounds occur in calf blood plasma, additional studies are needed to establish the exact nature and biological significance of these compounds.

SUMMARY

Blood plasma "lipid" phosphorus values in 4 young dairy calves were determined by extraction and precipitation procedures at weekly intervals during a 6-week period.

Trichloroacetic acid-precipitable phosphorus values compared favorably with those found in the alcohol-ether extracts. Lesser amounts of lipid phosphorus were found in the Skelly A-soluble and acetone-insoluble fractions; the latter were consistently lower. Small but significant quantities of residual phosphorus (present in Skelly A-soluble and water-soluble fractions) were found in all plasma samples. Trends in the Allen plasma fat values were similar to those for the alcohol-ether soluble phosphorus. Possible dietary effects on the plasma lipid phosphorus fractions are recognized.

REFERENCES

1. Allen, N.N. A simple volumetric method for determination of fat in blood plasma. Proc. Soc. Exptl. Biol. Med. 31:991. 1934.
2. Bloor, W.R. The oxidative determination of phospholipids (lecithin and cephalin) in blood and tissues. J. Biol. Chem. 82:273. 1929.
3. Boyd, E.M. The oxidative micro-estimation of blood lipids. Am. J. Clin. Path. 8:77. 1938.
4. Christensen, H.N. The contaminants of blood phospholipids. J. Biol. Chem. 129:531. 1939.
5. Folch, J., and D.D. Van Slyke. Nitrogenous contaminants in petroleum ether extracts of plasma lipids. J. Biol. Chem. 129:539. 1939.
6. Jacobson, N.L., J.H. Zaletel, and R.S. Allen. Effect of dietary lipid on the plasma lipid values in calf blood plasma. J. Dairy Sci. 36:832. 1953.
7. Kirk, E., I.N. Page, and D.D. Van Slyke. Gasometric micro determination of lipids in plasma, cells, and tissues. J. Biol. Chem 106:203. 1934.
8. Sinclair, R.G., and M.J. Dolan. The so-called ether insoluble phospholipids in blood and tissues. J. Biol. Chem. 142:659. 1942.
9. _____. The lecithin, cephalin, and sphingomyelin content of serum. 1. As indicated by the choline-phosphorus and nitrogen-phosphoru ratios. J. Biol. Chem. 174:343. 1948.
10. _____. The lecithin, cephalin, and sphingomyelin content of serum. 2. As indicated by the amino-nitrogen and nondiffusible phosphorus content after differential hydrolysis of the acetone-insoluble lipides. J. Biol. Chem. 174:355. 1948.
11. Stewart, C.P., and E.B. Hendry. The phospholipins of blood. Biochem. J. 29:1683. 1935.
12. Wittcoff, H. The Phosphatides. A C S Monograph Series, No. 112, Reinhold Publishing Corporation, New York, N.Y. 1951.
13. Zaletel, J.H., R.S. Allen, and N.L. Jacobson. Lipids in blood plasma of young dairy calves. J. Dairy Sci. 35:1046. 1952.
14. Zilversmit, D.B., and A.K. Davis. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J. Lab. Clin Med. 35:155. 1950.

IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 503-519

FACTORS AFFECTING GERMINATION OF KENTUCKY BLUEGRASS SEED[1]

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Various factors affecting germination of seeds have long been of interest to botanists. During recent years, seed analysts have become interested in these factors as they pertain to satisfactory laboratory germination tests. The effects of light, temperature and moisture have been studied extensively, while such factors as maturity at harvest, rate of drying, amount of after-ripening, time after harvest and storage conditions have received only limited attention.

Kentucky bluegrass (Poa pratensis L.) is one of the most difficult seeds to test for germination and obtain satisfactory results. For this reason there has been much controversy concerning the best method of germinating this seed. A number of workers (1, 2, 11, 13, 14, 25, 30, 36) consider light to be essential for best germination of bluegrass, while others (10, 16, 17, 28, 31, 32) consider light to be nonessential. The reason for some of the differences in response to light obtained by the above workers is explained by Hite (19, 20) and Jönson (21) who found the response of Kentucky bluegrass seed to light to be closely related to after-ripening of the seed. Well after-ripened seed showed little response to light, while non-after-ripened seed gave a higher germination with light. Cieslar (11) and Liebenberg (24) considered that the only effect of light was its heating action, while Laschke (23) concluded that light could not be replaced by high temperature. Bass (8) found that no one light intensity gave the highest germination for all samples of Kentucky bluegrass seed tested. He also found that light sensitivity decreased with increased time after harvest and that completely after-ripened seed germinated almost as well in darkness as in light. Bass (7) found that when light was filtered and used at a uniform 10 foot candles, some wave lengths were more effective than others in promoting germination of Kentucky bluegrass seed. The effectiveness of a given filter depended upon the maturity of the seed at harvest and/or the length of time after harvest that the test was made. Immature non-after-ripened seed germinated best with orange or green light and poorest with blue or red light. After-ripened and fully mature seed germinated almost equally well under all filters used.

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[1] Journal paper No. J-2105. Iowa Agricultural Experiment Station, Ames, Iowa. Projects No. 1083 and 1001. This is a portion of Doctoral thesis No. 953 entitled, "Effects of Light and Other Factors on Germination of Seed of Poa pratensis." Two typewritten copies of the complete thesis are on file at the Iowa State College Library.

Temperatures required for germination have been studied extensively with varying results. Several studies (12, 18, 19, 31) indicated that alternating temperatures of 20°C and 30°C, (20°C night and 30°C day) gave best results while others (1, 12, 27, 29, 30) thought that alternating temperatures of 15°C and 30°C were as good as, or better than, 20°C and 30°C alternating. Sprague (29) and Stebler (30) found that alternating temperatures of 10°C and 30°C gave satisfactory results. Another method which has received some attention is prechilling for five days at 5°, 10°, or 15°C. Bass and Isely (6) and Stebler (30) considered prechilling especially beneficial for freshly harvested seed, while Berry (9) was unable to obtain consistent results by prechilling. The Rules for Testing Seeds adopted by the Association of Official Seed Analysts (3) and the Rules and Regulations under the Federal Seed Act (33) formerly listed 20°C and 30°C alternating as the required temperatures for germinating Kentucky bluegrass seed; however, both sets of rules have been revised (4, 5, 34, 35) and the required temperatures are now 15°C and 30°C alternating, with prechilling at 10°C for 5 days as an alternate method.

Another important factor in seed germination is the moistening agent. Toole (31) found 0.2 per cent KNO_3 necessary for seed of Poa compressa but not for P. pratensis. Crosier and Cullinan (12) found KNO_3 to be of little value in germinating the average sample of Kentucky bluegrass seed. Andersen (1) obtained satisfactory germination of freshly harvested Kentucky bluegrass seed with either 0.1 per cent KNO_3 or water at 15°C and 30°C alternating temperatures when light was supplied during the high temperature period. Justice and Andersen (22) found 15°C and 30°C alternating temperatures with 0.1 per cent KNO_3 to give satisfactory germinations for both freshly harvested and after-ripened Kentucky bluegrass seed. Marth, Toole, and Toole (26) obtained best germination of Kentucky bluegrass seed from sod sprayed with 2,4-D, at 15°C and 25°C alternating with 0.2 per cent KNO_3 and light. The Official Rules for Seed Testing (3) formerly specified the use of 0.2 per cent KNO_3 for germinating Kentucky bluegrass; but this has now been changed to 0.1 per cent KNO (4, 5).

Some of the other factors affecting germination have received only limited attention. Hite (20) found that storage at 40°C increased the rate of germination of bluegrass seed during the first week of storage. He also found that maturity at harvest did not affect viability, within limits but after-ripening on the plant was more rapid than after-ripening during storage. Marth et al. (26) found no significant effects of storage on germination of Kentucky bluegrass seed.

Garman and Vaughn (15) warned that overheating should be guarded against during curing, as a temperature of 140°F (60°C) for a short while made seed worthless. They found that hand-stripped seed gave the best germination, and that the germination of immature seed was never as good as that of well matured seed, even when carefully cured.

Berry (9) found that bluegrass seed could be harvested when relatively immature and still germinate fairly well if sufficient time was allowed for maturation. The germination of a given seed lot fluctuated greatly in repeated tests, with abrupt increases and decreases. The time between harvest and maximum germination varied with maturity; the more mature the seed the shorter the period.

MATERIALS AND METHODS

The seed used in this study was hand-harvested from four locations n the vicinity of Ames, Iowa. Two collections were made, the first June 14-16) when the moisture content was approximately 35 per cent, nd the second (July 6-7) when the seed was fully mature and had a moisure content of approximately 8 per cent.

The lots of immature seed were divided into two parts for drying.)ne half was spread out in a large flat box in the laboratory at room temerature in front of an electric fan and was stirred several times a day o facilitate drying. The other half of each lot was put in a double paper ag and set on a table in the laboratory at room temperature to dry more lowly. The seed in the paper bags was stirred once a day while drying. The moisture content of the mature seed was the same after a week's lrying as it was when harvested, showing that it was air dry at harvest.

One half of the seed of each treatment, (a) immature seed rapidly lried, (b) immature seed slowly dried, and (c) mature seed, was stored a and b, July 7, and c, July 14) at 32°C, and the other half at 2°C. The relative humidity of the 32°C cabinet was between 15 and 20 per cent and hat of the 2°C cabinet was between 65 and 70 per cent. No moisture ests were made after the seed was stored.

Samples drawn for germination tests were prepared by hand-threshing nd blowing to remove the chaff and other inert material. For each germination test on each seed lot and treatment, two lots of 100 seeds each were counted on a vacuum counter and planted on moist quartz sand in petri dishes. Two methods were employed, which will be referred to as 1) regular and 2) prechill. The regular method consisted of placing the planted seed at alternating temperatures of 15°C and 30°C to germinate. The low temperature was maintained for 15 hours and high temperature for 9 hours each day. In the prechill method the moist seeds were kept at 10°C for 5 days, then they were transferred to temperatures alternating between 15°C and 30°C for germination. Tests were made by both the regular and prechill methods, with and without light, using either distilled water or 0.1 per cent KNO_3 as the moistening agent. All tests were of 28 days duration except where noted. The prechill period was not included in the germination time. The tests made in light received approximately 150 f.c. of fluorescent light during the high temperature period each day except Saturday afternoon and Sunday. The petri dishes for the dark tests were placed in light-tight wooden boxes covered to exclude light.

The germination percentages given in the tables represent the average germination for all lots and treatments at the indicated times. The number of seeds included in these averages were as follows: immature seed at harvest 800, 1, 2 and 3 weeks after harvest 1600, and 4 to 20 weeks after harvest 3200; mature seed at harvest and 1 week later 800, and 2 to 16 weeks after harvest 1600.

EXPERIMENTAL RESULTS

Effect of Maturity

The first germination tests were made as soon as the seed was harvested, and additional tests were made at various intervals thereafter. The data in Table 1 show that maturity affected the germination when the seed was planted immediately after harvest. The germination of the immature seed increased 59 per cent during the first week after harvest, but it was not until four weeks after harvest that maximum germination was obtained. The mature seed gave its highest germination one week after harvest. This date coincided with the time when the immature seed gave its highest germination. For both maturities there was a decrease in germination after the peak was reached, followed by a slight increase.

TABLE 1

Percentage germination of immature and mature Kentucky bluegrass seed tested by the regular method with light at the indicated intervals after harvest.

Weeks after harvest	Immature seed	Weeks after harvest	Mature seed
0	20.4		
1	79.5		
2	88.0		
3	87.3	0	89.8
4	92.3	1	92.1
7	78.3	2	86.5
10	82.3	6	83.6
15	83.8	11	86.1
20	84.8	16	87.5

These data are not complete enough to show the whole pattern of loss of dormancy during maturation of Kentucky bluegrass seed. However, the fact that the highest percentages of germination of both the immature and mature seed were obtained with tests planted at the same time seems to indicate that dormancy is closely associated with maturation of the seed. The germination tests made on the immature seed at intervals during drying may well indicate the general pattern of rate of dormancy loss in the seed while still on the plant.

The data show also that the germination of given lots of seed may fluctuate from time to time, confirming the findings of Berry (9). The mature seed showed less variation and maintained a higher average germination than did the immature seed.

Effect of Storage

The effects of storage temperature and storage time on the germination of Kentucky bluegrass seed are given in Table 2 for both the mature and immature seed. A statistical analysis of the data is given in Table 8.

TABLE 2

Effect of storage temperature and storage time on percentage germination of immature and mature Kentucky bluegrass seed tested by the regular method with light.

Weeks of storage	Immature		Weeks of storage	Mature	
	32°C	2°C		32°C	2°C
0	87.3	87.3	0	92.1	92.1
1	94.7	89.6	1	88.1	84.9
3	75.7	81.2	4	86.3	80.9
6	78.6	85.9	10	87.6	84.6
11	81.0	85.4	14	90.5	84.4
16	81.1	87.2	Average	88.9	85.4
Average	83.1	86.1			

One week of storage gave maximum germination for the immature seed. The maximum germination of the first week was followed by a sharp drop, especially for the seed stored at the high temperature, and a subsequent increase. The seed stored at 32°C dropped 19 per cent and later increased only 6 per cent, indicating a possible loss of viability, whereas the decrease for the low temperature seed could be attributed to secondary dormancy, since the germination of this seed later increased and equaled the maximum obtained after one week of storage.

Although the differences in germination due to storage temperature were not large, the data indicated that the low storage temperature was best for the immature Kentucky bluegrass seed used in this experiment. Maximum germination of the mature seed was obtained at the time of storage, followed by fluctuating results. In contrast to the immature seed, the mature seed stored at 32°C gave the highest germination.

Effect of Prechilling

Tests were made on immature and mature seed by the regular and prechill methods. The results of these tests (part of Table 3) were not significantly different except for the immature seed tested at harvest, 8 replicates of 100 seed each, which gave a difference of 54 per cent in favor of the prechill method. For the mature seed there were no significant differences between the two methods.

Effects of Light and Moistening Agent

The results of tests made with and without light at various intervals after harvest of both the immature and mature seed tested by the regular and prechill methods are given in Table 3. No lots of immature seed germinated better in the dark than in light, although a few gave essentially the same results. One week after harvest there was a difference of 70.6 per cent in favor of light with the regular method. As after-ripening progressed, the sensitivity of the immature seed to light decreased; the prechill method showed a more rapid decrease in sensitivity to light than the regular method. However, the prechill test made in the dark 15 weeks after harvest gave an unexpectedly poor result for which no ready explanation is available.

TABLE 3

Percentage germination of Kentucky bluegrass seed planted in light and darkness using 0.1 per cent KNO_3 as moistening agent.

Weeks after harvest	Regular		Prechill	
	Light	Dark	Light	Dark
		Immature seed		
0	20.4	0.4	74.6	11.0
1	79.5	8.9	85.5	34.9
2	88.0	27.2	87.5	45.9
3	87.0	25.2	87.0	64.7
4	92.3	75.5	93.0	89.3
7	78.3	76.5	78.8	75.1
10	82.3	75.9	81.3	81.4
15	83.8	80.5	81.0	67.3
20	84.8	74.5	81.8	76.1
Average	77.4	49.4	83.4	60:0
		Mature seed		
0	89.8	67.3	89.9	81.0
1	92.1	85.1	91.4	90.4
2	86.5	86.5	87.3	88.3
6	83.6	83.4	84.4	84.9
11	86.1	87.1	87.5	88.3
16	87.5	86.9	87.2	86.0
Average	87.6	82.6	87.9	86.5

A few tests were made by the four methods mentioned in the preceding paragraph on both immature and mature seed using distilled water as the moistening agent. The results are given in Table 4.

TABLE 4

Percentage germination of Kentucky bluegrass seed planted in light and darkness using water as moistening agent (Av. of 8, 16, or 32 x 100 seeds).

	Weeks after harvest							
	Immature seed					Mature seed		
	1	2	3	20	Ave.	0	16	Ave.
Regular Method								
Light	52.3	71.3	82.3	83.4	72.7	83.0	85.3	84.2
Dark	2.6	3.3	3.3	46.5	13.9	23.5	79.0	51.3
Prechill Method								
Light	76.5	78.0	84.3	80.2	79.8	87.0	88.8	87.9
Dark	10.8	7.3	46.0	52.3	29.1	70.0	90.0	80.0

These data show that there was a greater response to light with distilled water as the moistening agent than with 0.1 per cent KNO_3 (Table). especially in tests on the immature seed. The germination of the mmature seed 20 weeks after harvest by the regular method was approximately the same for both moistening agents (Table 3 and 4) with light, ut in the dark there was a difference of 28 per cent in favor of 0.1 per ent KNO_3. The prechill test gave results similar to those for the regular method. The germination, with light, of mature seed tested at harest was approximately the same by all methods; however, there were ide differences between the dark tests.

The data in Table 3 and 4 show light and 0.1 per cent KNO_3 to have een about equally significant in promoting germination of Kentucky bluerass seed and that one could partially substitute for the other. However, est results were obtained when they were used in combination.

Effects of Maturity, Drying Time, and Germination Method

The results of germination tests, by both the regular and prechill nethods, with and without light, made during the periods of curing of oth the immature and mature seed, are given in Table 5.

Statistical analyses (Table 6) shows that when time alone was considred, an F value significant at the 1 per cent level was obtained for both mmature and mature seed. The same was true for the different germiation methods. The interaction of germination methods was significant t the 1 per cent level for the mature seed and at the 5 per cent level for he immature seed. The interaction of germination method and time was ighly significant for both mature and immature seeds. The germination f the immature seed 3 weeks after harvest was essentially the same, by he regular and prechill methods with light, as that for the mature seed t harvest (planted the same time), indicating that the light sensitiveness t both was the same. However, they were not necessarily in the same

TABLE 5

Percentage germination during curing of Kentucky bluegrass seed harvested at two stages of maturity.

Germination method	Weeks after harvest							
	Immature seed					Mature seed		
	0	1	2	3	Ave.	0	1	Ave.
Regular								
Light	20.3	79.4	87.9	87.3	68.7	89.7	92.1	90.9
Dark	0.4	9.0	27.2	25.2	15.5	67.1	85.1	76.1
Prechill								
Light	74.6	84.9	86.9	87.1	83.4	87.9	91.4	89.7
Dark	11.0	35.1	45.8	64.4	39.1	81.9	90.4	86.2

TABLE 6

Analysis of variance of germination data for immature and mature Kentucky bluegrass seed before storage.

	Immature seed		
	Degrees of freedom	Mean square	F
Samples	3	185.78	0.063
Drying rate	1	236.53	0.80
Time	3	10,015.07	127.27**
Time x drying rate	3	122.09	1.55
Germination method	3	29,542.86	321.58**
Official vs. chill	1	11,704.50	127.40**
Light vs. dark	1	76,293.94	830.45**
Germ. method x drying rate	3	340.07	3.70*
Germ. method x time	9	1,348.37	14.67**
Germ. method x drying rate x time	9	78.13	0.85

	Mature seed		
Samples	3	5.80	1.09
Time	1	461.32	87.04**
Germination method	3	382.11	18.98**
Official vs. chill	1	187.70	9.32**
Light vs. dark	1	745.95	37.05**
Interaction	1	212.69	10.56**
Germ. method x time	3	115.65	5.47**

*Significant at the 5 per cent level
**Significant at the 1 per cent level

stage of after-ripening, as their dark germinations were different. The mature seed gave a much higher dark germination than the immature seed, indicating that after-ripening takes place more rapidly during normal maturation and drying on the plant than during drying of immature seed harvested green.

Interaction of Drying Rate and Germination Method

Seed harvested at 35 per cent moisture was dried at two rates to determine the effect of drying on germination. The difference due to drying rate alone was not significant.

The time after harvest at which the germination test was made was highly significant. The high F value was, without question, due to dormancy at harvest and loss of dormancy during drying. Although time alone produced highly significant differences in germination, the difference due to the interaction of time and drying rate was not significant. The F value for the interaction of germination method with drying rate and with time was not significant.

Germination methods gave a highly significant F value. When germination methods were separated into regular vs. prechill and light vs. dark, both comparisons gave F values significant at the 1 per cent level. Their interactions produced an F value significant at the 5 per cent level.

The drying rate had little effect on either the regular or prechill results when light was supplied, but for the dark tests there were considerable differences between the two drying rates. The rapidly dried seed germinated slightly better in the dark than did the slowly dried, except in those tests made two weeks after harvest by the regular method.

Effects of Storage Temperature, Storage Time, and Germination Methods

One-half of each lot of seed was stored at 32°C and the other at 2 °C. Germination tests were made by both the regular and prechill methods with and without light, at various times after storage. The germination results for both immature and mature seed are given in Table 7 and statistical analyses are given in Table 8.

The data indicate that storage temperature was not instrumental in producing significant differences in germination during storage. The significant differences obtained for immature seed were due to length of storage, germination method (light vs. dark), and interaction of the various factors. There were no significant differences for the mature seed.

Germination Rate

Commercial lots of Kentucky bluegrass seed as a rule show over 95 per cent of their total germination during the first 17 days of the test. The rates of germination, when tested during drying, of the immature Kentucky bluegrass seed used in the preceding experiments are given in Fig. 1, while the rates when tested during storage are given in Fig. 2. In these curves, germination at the indicated time (month and day as 6/15, 6/23, etc.) is plotted as a percentage of total final germination by the

regular method. The total germination used was not necessarily the maximum obtainable but was that obtained during the experimental time of 28 to 43 days. Since there were no differences in the curves for the two drying rates, only those for the slowly dried seed are given. For ease of comparison, both the light and dark tests are given in the same figure.

TABLE 7

Percentage germination of immature and mature Kentucky bluegrass seed stored at 2°C and 32°C.

Weeks of storage	Regular				Prechill			
	Light		Dark		Light		Dark	
	Storage Temperature							
	2°C	32°C	2°C	32°C	2°C	32°C	2°C	32°C
	Immature Seed							
0	87.3	87.3	25.2	25.2	87.1	87.1	64.4	64.4
1	89.6	94.7	68.3	82.6	92.1	93.8	88.1	91.7
3	81.2	74.7	79.9	72.8	80.9	76.5	76.0	74.1
6	85.9	78.6	84.4	77.3	84.3	78.4	84.1	78.1
11	85.4	81.0	83.6	77.3	85.7	75.8	62.4	71.1
16	87.2	81.1	74.8	74.1	86.5	78.5	76.9	75.1
Av.	86.1	82.9	69.0	68.2	86.1	81.7	75.3	75.7
	Mature Seed							
0	92.1	92.1	85.1	85.1	91.4	91.4	90.4	90.4
1	84.9	88.1	86.6	86.3	88.1	86.4	89.1	87.5
4	80.9	86.3	81.4	84.3	82.5	86.3	84.9	84.8
10	84.6	87.6	84.5	89.6	85.4	89.5	85.5	91.0
15	84.4	90.5	85.1	88.6	86.8	87.6	84.3	88.6
Av.	85.4	88.9	84.5	86.8	86.8	88.2	86.8	88.4

The rate of germination after 16 weeks storage is included in Fig. for comparison with the rate at harvest and during drying. During drying, the rate of germination, when tested by the regular method, increased gradually. At harvest (6/15) the dark test showed no germination in 28 days although it did eventually germinate 65 per cent in 43 days. The light test gave one-fifth of its 87 per cent germination during the first 28 days. After one week (6/23) of drying the rate for the dark test increased about 15 per cent in 28 days, while that of the light test increased 70 per cent. When the dried seed was stored (7/8) the dark test gave 15 per cent of its total germination in 17 days while the light test gave 92 per cent in the same period. After 6 weeks storage (not shown) the light test gave 95 per cent of its total germination in 9 days, and the dark test 84

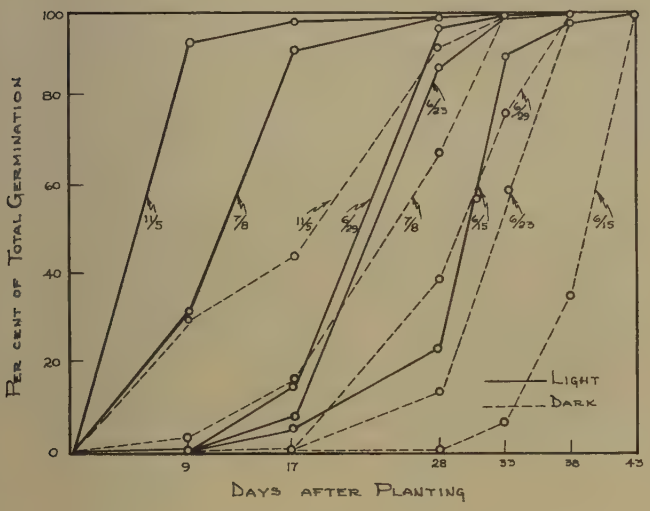

Fig. 1. Rate of germination of slow dried immature Kentucky bluegrass seed tested by the regular method on the indicated dates during drying.

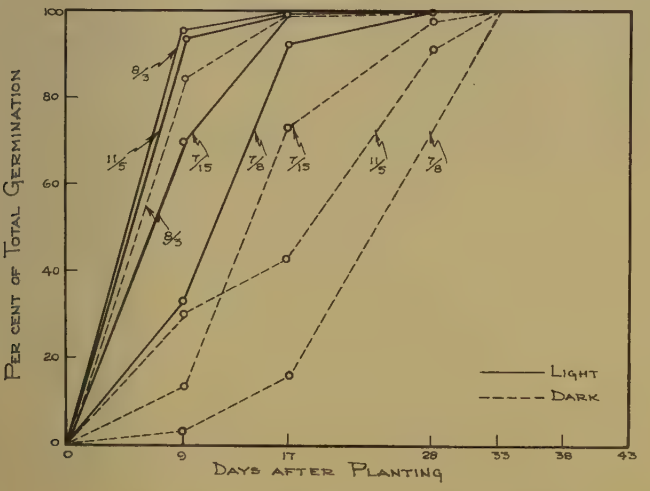

Fig. 2. Rate of germination of slow dried immature Kentucky bluegrass seed stored at 2°C, tested by the regular method on the indicated dates during storage.

TABLE 8

Analysis of variance of germination data for immature and mature Kentucky bluegrass seed during storage.

	Immature seed		
	Degrees of freedom	Mean square	F
Samples	3	894.94	1.69
Drying rate	1	605.00	1.18
Storage temperature	1	529.56	0.65
Storage temperature x drying rate	1	109.27	0.15
Storage time	4	1,139.02	16.36**
Storage time x drying rate	4	48.12	0.68
Storage time x storage temperature	4	394.20	5.66**
Storage time x drying rate x storage temperature	4	9.57	0.14
Germination method	3	984.89	55.27**
Official vs. chill	1	7.20	0.40
Light vs. dark	1	2,922.16	163.92**
Germ. meth. x drying rate	3	161.31	6.53**
Germ. meth. x storage temperature	3	131.15	7.36**
Germ. meth. x storage time	12	355.98	19.97**
Germ. meth. x storage temperature x drying rate	3	7.59	0.42
Germ. meth. x storage time x drying rate	12	49.58	2.78**
Germ. meth. x storage time x storage temperature	12	81.74	4.58**
Germ. meth. x storage time x drying rate x storate temperature	12	9.76	0.54

	Mature seed		
Samples	3	881.62	11.02*
Storage temperature	1	241.99	3.02
Storage time	3	87.97	2.66***
Storage temperature x storage time	3	33.60	1.01
Germination method	3	9.43	1.30
Official vs. chill	1	26.28	3.62***
Light vs. dark	1	0.01	0.00
Germ. meth. x storage temperature	3	10.98	1.51
Germ. meth. x storage time	9	5.33	0.73
Germ. meth. x storage temperature x storage time	9	8.22	1.13

* Significant at the 5 per cent level
** Significant at the 1 per cent level
*** Almost significant

er cent. After 17 weeks the light test was unchanged but the dark test, or some unexplained reason, was slow again; only 30 per cent in 9 days.

No curves are given to show the germination rates by the prechill method since they followed the same pattern as those for the regular method except that the germination rate was increased more rapidly by the prechill method.

The rates of germination with distilled water as the moistening agent were similar to those with 0.1 per cent KNO_3 only somewhat lower for all tests made.

DISCUSSION

The problem of obtaining satisfactory germination tests on Kentucky bluegrass seed has been studied with particular emphasis on the effects of light, temperature and moistening agent, and their relation to other factors, such as maturity at harvest, drying rate, time after harvest, storage conditions, and length of storage. The conclusions are that maturity at harvest and time after harvest are most important, and that the effects of the other factors are directly related to these two. There is also interaction among the various factors.

Tests on hand-harvested seed showed that the moistening agent is most important during the first few months after harvest for seed harvested while still immature. When freshly harvested, immature seed was planted with water as moistening agent and germinated in light, a lower germination was obtained than when 0.1 per cent KNO_3 was used. The same seed tested in darkness gave very little germination by the regular method, regardless of the moistening agent. When the seed was prechilled 5 days at 10°C, then transferred to 15°C and 30°C alternating, increased germination was obtained with either distilled water or 0.1 per cent KNO_3 and light. These results show the complexity of the problem of obtaining uniform germination results on a given lot of Kentucky bluegrass seed tested simultaneously in more than one seed laboratory. If the germination method used in the various laboratories is not the same, the germination results will not necessarily be the same.

Prechilling, plus 0.1 per cent KNO_3 and light, is usually effective in breaking the dormancy of Kentucky bluegrass seed. However, not all samples respond favorably to prechilling, and, under certain physiological conditions of the seed, the prechill test may give decreased germination. For this reason it seems advisable to test all samples of Kentucky bluegrass seed by both the regular and prechill methods.

Seed that was completely mature when harvested showed less dormancy, germinated more rapidly, and maintained a higher germination throughout the tests than did the seed harvested while still immature. (Fig. 3). This suggests that commercial harvesters of Kentucky bluegrass would do well to let the seed ripen more on the plant than is common practice at the present time.

The rate of germination of Kentucky bluegrass seed is, in general, a good indicator of the physiological condition of the seed. The rate of germination increased with time after harvest until over 90 per cent of the total was obtained in 9 days with light and 0.1 per cent KNO_3.

The problem of seed analysis is made more difficult by the fact that

the analyst may know nothing about the previous treatment of the sample. The present study has shown that the previous treatment will affect the germination of the seed, especially with regard to the best germination method to use. If the analyst knew the age of the seed, maturity at harvest, and storage conditions, he would be in a better position to obtain the maximum germination in the minimum time by selecting the proper method for testing.

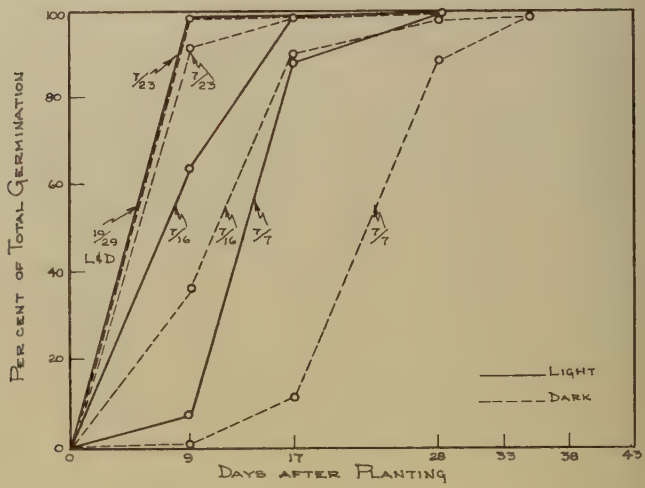

Fig. 3. Rate of germination of mature Kentucky bluegrass seed at harvest (7/8) and on the indicated dates during storage at 2° C, tested by the regular method.

There are several factors not included in this study which may be important in arriving at the ultimate solution to the problem of obtaining satisfactory germination results for Kentucky bluegrass seed. It is possible that environmental factors, such as temperature and moisture supply during the development and maturation of the seed, may affect its germination. Another factor which may be important is the soil fertility and the available supply of some of the trace elements. The geographical source of the seed may also be important.

SUMMARY AND CONCLUSIONS

1. The most important factor affecting the germination of Kentucky bluegrass seed, regardless of germination method or storage condition, was the maturity of the seed when harvested.

2. There was a certain amount of interchangeability of effect of light, moistening agent, and germination method (regular and prechill) depending upon the physiological condition of the seed when tested.

3. Kentucky bluegrass seed harvested while still immature was dormant and very sensitive to light, temperature, and moistening agent when planted immediately after harvest, but as after-ripening progressed it lost its dormancy and sensitivity to these factors.

4. Seed that was mature when harvested was less sensitive to germination conditions, and two weeks after harvest there were no differences in results obtained by the various methods.

5. Immature, hand-harvested seed was dried at two rates, slowly and rapidly. The differences in germination due to drying rate were not significant.

6. Two storage temperatures, 2°C and 32°C, were used but they did not produce significant differences in germination. The immature seed stored at 2°C germinated slightly higher than that stored at 32°C, while the mature seed germinated slightly higher when stored at 32°C. All lots of seed attained a maximum germination of over 90 per cent. The peak germination was followed by a decline during the second and third week of storage with a subsequent increase.

7. Significant differences in germination due to the interaction of drying rate, storage temperature, length of storage, and germination method were obtained for the immature but not for the mature seed.

8. The rate of germination increased with length of time after harvest, regardless of germination method. However, it increased most rapidly with the prechill method using 0.1 per cent KNO_3 and light. It also increased faster for the mature seed than for the immature seed.

The author wishes to express his appreciation to Professors W. E. Loomis and Duane Isely for advice and assistance during both the study and preparation of the manuscript. Thanks are due Professor P. G. Homeyer for help with statistical analysis, and members of the Seed Laboratory staff for technical assistance.

REFERENCES

1. Andersen, A.M. Germination of freshly harvested seed of Kentucky bluegrass. Proc. Assoc. Off. Seed Anal. 33:96-98. 1941.
2. _____. Some factors influencing the germination of Poa compressa L. Proc. Assoc. Off. Seed Anal. 37:134-143. 1947.
3. Association of Official Seed Analysts. Rules for testing seeds. Proc. Assoc. Off. Seed Anal. 35:17-42. 1944.
4. _____. Rules for testing seeds. Proc. Assoc. Off. Seed Anal. 37: 39-75. 1947.
5. _____. Rules for testing seeds. Proc. Assoc. Off. Seed Anal. 39: 23-57. 1949.
6. Bass, L., and D. Isely. A proposed change in the rules for germination of Kentucky bluegrass. News Letter Assoc. Off. Seed Anal. 23(1):19-22. 1949 (Mimeo. report)
7. _____. Effect of wave length bands of filtered light on germination of seeds of Kentucky bluegrass (Poa pratensis). Iowa Acad. of Sci. 57: 61-71. 1950.
8. _____. Effect of light intensity and other factors on germination of seeds of Kentucky bluegrass (Poa pratensis L.). Proc. Assoc. Off. Seed Anal. 41:83-86. 1951.

9. Berry, J.L. Seasonal maturity, dormancy and germination of seeds of bluegrass, timothy and oats, Unpublished Masters Thesis. Iowa State College Library. 1944.
10. Brown, E. Germination of Kentucky bluegrass. U.S.D.A. Off. Agr. Exp. Sta. Bull. 115. pp. 105-110. 1920.
11. Cieslar, A. Untersuchung über den Einfluss des Lichtes auf die Keimung der Samen. Forsch. a.d. Gebiet. Agrik. Physik. 6:270-295. 1883.
12. Crosier, W., and B. Cullinan. Some observations in the germination of grass seed. Proc. Assoc. Off. Seed Anal. 33:69-74. 1941.
13. Fryer, J.R. The influence of light and fluctuating temperature on the germination of Poa compressa L. Sci. Agr. 2:225-230. 1922.
14. Gadd, I. Über Methoden zur Hebung mangelnder Keimreife in der Samenkontrollarbeit. Proc. Inter. Seed Test. Assoc. 11:96-107. Eng. trans. 11:108-118. 1939.
15. Garman, H., and E.C. Vaughn. The curing of bluegrass seeds as affecting their viability. Ky. Agr. Exp. Sta. Bull. 198. 1916.
16. Gassner, G. Untersuchungen über die Wirkung von Temperatur und Poa Arten. Zeitschr. f. Bot. 23:767-837. 1930.
17. Goss, W.L. The germination of Kentucky bluegrass seed in blotters, bell-jars and petri dishes. Proc. Assoc. Off. Seed Anal. 14/15: 119-120. 1923
18. Harrington, G.T. Use of alternating temperature in the germination of seeds. J. Agr. Res. 23:295-332. 1923.
19. Hite, B.C. Forcing the germination of bluegrass. Proc. Assoc. Off. Seed Anal. 11:53-58. 1919.
20. _____. Effect of storage on the germination of bluegrass seed. Proc. Assoc. Off. Seed Anal. 14/15:97. 1923.
21. Jönsson, B. Jaktagelsen of verLjusets Betydelse for Frens Groning. Lunds Univ. Arsskr. 29:40-47. 1893. (original not seen; cited by Gardner, Bot. Gaz. 71:250. 1921).
22. Justice, O.L., and A.M. Andersen. Germination of Kentucky bluegrass at two alternating temperatures. News Letter Assoc. Off. Seed Anal. 20(1):10-12. 1946. (Mimeo report)
23. Laschke, W. Einige vergleichende Untersuchungen über den Einfluss des Keimbettes sowie des Lichtes auf die Keimung verschiedener Sämereien. Landw. Versuchsstat. 65:295-300. 1907.
24. Liebenberg, A. Über den Einfluss intermittierender Erwärmung auf die Keimung von Samen. Bot. Centralbl. 18:21-26. 1884.
25. Maier, W. Untersuchungen zur Frage der Lichtwirkung auf die Keimung einiger Poa Arten. Jahrb. f. Wiss. Bot. 77:321-392. 1932-1933.
26. Marth, P.C., V.K. Toole, and E.H. Toole. Yield and viability of Kentucky bluegrass seed produced in sod areas treated with 2,4-D. J. Am. Soc. Agron. 39:426-429. 1947.
27. Moringa, T. Effect of alternating temperature upon the germination of seeds. Am. J. Bot. 13:141-158. 1926.
28. Nelson, A. The germination of Poa spp. Ann. Appl. Biol. 14:157-174. 1927.
29. Sprague, V.G. Germination of freshly harvested seeds of several Poa species and of Dactylis glomerata. J. Am. Soc. Agron. 32: 715-721. 1940.

30. Stebler, E.G. Über den Einfluss des Lichtes auf die Keimung. Bot. Centralbl. 7:157-158. 1881.
31. Toole, E.H. A preliminary report on bluegrass germination. Proc. Assoc. Off. Seed Anal. 14/15:119. 1923.
32. _____. Changes in "Rules for seed testing". Proc. Assoc. Off. Seed Anal. 17:35-38. 1925.
33. U.S. Agricultural Marketing Service. Rules and regulations under the Federal Seed Act. Service and Regulatory Announcements No. 156. March 1940.
34. _____. Rules and regulations under the Federal Seed Act. Service and Regulatory Announcements No. 156. Revised with Amendments February 1946.
35. _____. Rules and regulations under the Federal Seed Act. Service and Regulatory Announcements No. 156. Reprinted with Amendments July 1950.
36. Waldron, C.H. Notes on germination of Kentucky bluegrass. Proc. Assoc. Off. Seed Anal. 12/13:14-15. 1921.

IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 521-586

NOXIOUS WEED SEEDS I.[1]

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Noxious weeds are plants which are capable of disrupting ordinary farming operations and resisting measures for control or eradication. These plants are our worst weeds.

Most of the various kinds of weeds, noxious and otherwise, have extended themselves far beyond their original natural areas and are still spreading, some at an alarming rate. The most important agency responsible for their dissemination is crop seed--nearly all agricultural seed lots contain weed seeds in greater or lesser amounts. In an attempt to limit the further spread of the worst of weeds, seed laws have designated the seeds of these weeds as noxious, and prohibit or restrict the sale of agricultural seed containing such weed seeds.

Weed Seed Identification in Seed Analysis

A knowledge of the identity of weed seed contaminants is useful in determining the quality of commercial seed. Seed analysts, in the course of purity testing, usually identify most of the incidental weed seeds, However, the identification of common weed seeds is not always an immediate necessity, and if "unknowns" are encountered, they may possibly be set aside for study at a future date. It is essential that all noxious weed seeds be identified correctly.

The seeds of some weeds are distinctive in appearance and are not readily confused with those of other kinds. Once the analyst has studied such seeds and familiarized himself with their appearance, their future identification is relatively simple. The seeds of many weeds, however, closely resemble those of other plants and a concise knowledge of the differential characters is desirable if the seed concerned is to be identified with certainty. With reference to noxious weed seed identification, the analyst must not only know what the noxious weed seeds look like, but must be equally familiar with similar seeds, and clearly understand the differences among them. In some cases, the similarities are such that maturity, degree of processing, area of occurrence, etc., must be given due consideration before a definite conclusion can be reached.

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[1] Journal Paper No. J - 2277 of the Iowa Agricultural Experiment Station, Project 1083, Ames, Iowa. This study was financed, in part, with funds appropriated under the Research and Marketing Act of 1946 and was carried out in cooperation with the Grain Branch Production and Marketing Administration, United States Department of Agriculture.

Literature

Seeds of agricultural and weedy species are illustrated in currently available general treatments (Hillman and Henry, 1935; Wright, 1950; U.S.D.A., 1952). Investigations of a number of difficult or important groups have also been reported, studies by Musil predominating (see references). Isely (1949) and Bellue (1949) published illustrated treatments of the noxious weed seeds of their respective states (Iowa and California), comparing the noxious seeds with others (common weeds and crops) most apt to be confused with them.

Scope of the Present Study

Subsequent to the appearance of the above-cited papers on the noxious weed seeds of Iowa and California, numerous analysts have suggested the desirability of applying this approach on a broader basis so as to include weed seeds declared noxious in additional areas. The present investigations attempt to fill such a need. Patterned after those of the above authors, they deal with those weed seeds declared noxious by law in the United States, Canada, and Alaska.[1] Principal emphasis is placed upon the differential characters of the noxious weed seeds and those of other weeds and crops which are similar in appearance. Supplementary information relating to the legal status, distribution, and importance of the noxious weeds is provided to broaden the usefulness of the treatment.

This publication represents the first part of this investigation. The weeds treated include the noxious representatives of the sedge, grass, lily, nettle, and smartweed families--the preponderance are grasses. The remaining groups will be described in subsequent publications.

Descriptive Treatment of Noxious Weed Seeds

The main text of the present paper consists of descriptions and illustrations of noxious weed seeds, and other weed and crop seeds with which they are readily confused. The sequence of groups treated is: Cyperaceae, Gramineae, Liliaceae, Urticaceae, and Polygonaceae (usual botanical order except that sedges precede grasses). Genera are treated alphabetically within each family. Readers unfamiliar with this sequence can locate individual weed seeds in the indices at the end of the bulletin.

The individual species are treated under the following captions:

Status under law. The seed laws declaring the seed seed noxious are enumerated. The laws considered are those of each of the 48 states in the United States, the Federal Seed Act,[2] the Canadian Seeds Act, and

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1 Because of the large diversity in weeds declared noxious by the various state seed laws in the United States, the number of noxious weeds in this country exceeds one hundred, and includes, with very few exceptions, all of the noxious weeds of the total area as above designated.

2 As applied to imported seed. The Federal Seed Act requirements concerning seed shipped in interstate commerce are that the seed must comply with noxious weed seed requirements of the state to which it is shipped.

the Alaskan Seed Law. The nature of the legislation applying to the weed is characterized by enumerating the subject states under the headings: primary noxious, restricted, secondary noxious. These are defined as follows: Primary noxious--The weed seed is prohibited, with or without tolerance. Restricted--Occurrence of the weed seed is restricted or limited; sale of agricultural seed containing weed seeds in excess of certain maximums (e.g. one hundred per ounce, two hundred per pound) is prohibited; rate of occurrence of the weed seed must be declared on the label. Secondary noxious--The occurrence of the noxious weed seeds must be declared upon the analysis label, but sale of agricultural seed containing such seed is subject to no restrictions other than those applying to total percentage by weight of all weed seeds.[1]

It should be borne in mind that terminology employed in state laws is diverse, and does not in many cases coincide with the above. This classification was devised to give the analyst a summary picture of the legal status of noxious weeds. If details for specific states are required, it is desirable to refer to the laws themselves. Summaries of noxious weed seed requirements are published annually in the Seed Trade Buyers Guide (1952), and by the Federal Seed Act Division (1951).

Since state laws may be subject to change from time to time, analysts wishing to keep this check list up to date can make deletions or write in additions as such changes come to their attention.

How produced. The fruit and associated plant structures are described briefly, Since, in many cases, the seed of common terminology is botanically a seed plus accessory enveloping structures, the word "seed" as applied to the weed in question is defined.

Size. Length and width are given in millimeters or centimeters.

Description. Brief descriptions place emphasis on characters which contrast the noxious weed seed with other seeds resembling it.

Seeds with which the weed seed in question may be confused. Similar seeds are enumerated and contrasted with the noxious weed seed in question.

Detection and diagnosis. Special problems relating to the determination of the noxious weed seed in agricultural seed are discussed. This section is included only for a few of the more difficult noxious weed seeds, or those presenting special problems not adequately covered under the above headings.

Distribution and importance as a weed. A brief summary of the occurrence and characteristics of the weed is presented.

Occurrence in agricultural seed. Insofar as information is available, an attempt is made to indicate the specific crops or kind of seed with which the weed seed is generally associated. In some cases a general statement is made as to the relative frequency of occurrence; likewise, if it is known that source of seed may affect the presence or absence of the weed seed, this is indicated.

Literature. Other currently available or comprehensive treatments or illustrations of the weed seeds are cited.

Illustrations. The noxious weed seed and the crop or common weed seeds with which it is compared are illustrated. Figure references are

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[1] Varies from 1-5 per cent in various states.

given in the text following the name of the weed; the bold-face number or numbers indicate illustrations of the weed seed under discussion; the following figures refer to illustrations of seeds of other weeds or crops with which the noxious weed seed is compared.

GLOSSARY

The employment of many of the technical or semi-technical terms herein defined is also discussed in the text, particularly in the general section characterizing grass seeds (page 529).

Achene. A seed-like, one-seeded indehiscent fruit. Popularly, most achenes are called seeds. Examples: The seed-like fruits of smartweeds, docks, and sedges.

Axil. The angle between a leaf, or bract, and the stem.

Biconvex. Convex on both sides; lens-shaped.

Bract. A reduced leaf-like structure in the axil of which a flower or flower cluster arises. The bracts, or assemblages of bracts of some plants may completely hide the flower from external view.

Bulblets. Small, bulb-like, vegetatively produced propagules.

Callus. The hardened area or ring at the basal extremity of the lemma on grass seeds.

Calyx. The outer, usually greenish hull of flower parts; the sepals collectively.

Cancellate. With a brick-like, finely bumpy, or porous appearance.

Caryopsis. The one-seeded indehiscent fruit of the grasses. Synonymous with grain.

Coriaceous. Hard, leathery in texture.

Corolla. The petals of the flower, usually the conspicuous colored portion of the flower.

Dentate. Toothed.

Disarticulate. To break or separate especially at a specialized joint. The term is used in regard to the separation, one from another, of grass florets, or of the spikelet from the parent plant.

Endosperm. A specialized food storage tissue within many kinds of seeds. Grass caryopses and smartweed achenes contain extensive endosperm. In bean seeds, on the other hand, the stored food is almost entirely within the cotyledons; the endosperm is, for all practical purposes, absent.

Floret. The flowering unit within a grass spikelet, consisting of a flower and its enveloping lemma and palea. The grass seed of common terminology is, in many cases, a mature floret; i.e. the grain enclosed within the lemma and the palea.

Geniculate. Abruptly bent.

Glabrous. Without hairs; smooth.

Glume. One of outer set of hulls in a grass spikelet.

Grain. The one-seeded, indehiscent fruit of the grasses. Synonymous with caryopsis.

Hulled (seed or grain). With the hulls (lemma and palea, glumes or calyx removed.

Imbricate. Overlapping; shingle-like.

Indehiscent. Not splitting open at maturity. Referring particularly to one-seeded fruits, achenes or grass caryopses.

Inflorescence. The flower cluster or clusters on a plant.
Keel bristles. Bristles or hair frequently found along the palea keels.
Lacerate. Irregularly torn or toothed; with long, narrow, irregularly disposed teeth.
Lanceolate. Elongate and pointed towards the tip; lance-shaped.
Lemma. The hull or bract in a grass spikelet which bears a flower in its axil. The lemma is one of the two hulls which surround the grain of many grass "seeds". (See discussion of the grass family in the text for further details.)
Palea. One of the two bracts or hulls within the grass spikelet which surrounds the flower; in the grass "seed", one of the hulls or coverings around the grain. (See the discussion of the grass family in the text for further details.)
Palea keel. A longitudinal fold or keel near the lateral margin (one to each edge) of the palea of many kinds of grasses.
Panicle. A compound, branched inflorescence bearing stalked flowers or flowering units; examples, the panicles of brome grass or of oats.
Papillate. With small rounded projections.
Pericarp. Fruit coat or fruit tissue.
Pistillate. Possessing only pistils, female. May refer either to a pistillate flower or to a plant.
Plano-convex. Convex on one side and flat on the other; turtle-shaped.
Rachilla. A short stalk arising at the base of many grass seeds and directed upwards.
Reticulate. Netted; with a network-like appearance.
Rhizome. A horizontally spreading underground stem.
Rugose. Roughened by lines or ridges.
Scaberulous. With very short, rough hairs.
Scabrous. Rough or harsh to the touch, usually from short stiff hairs.
Sessile. Not stalked. Lacking a petiole or pedicel.
Spike. An unbranched inflorescence in which the individual flowers or flowering units are sessile: example, spikes of wheat or ryegrass.
Spikelet. The flowering unit characteristic of the grasses; consisting of an outer set of hulls, glumes, above which one to several flowers are borne in the axils of secondary hulls, lemmas. (See discussion of the grass family for a fuller interpretation of the spikelet.)
Stolon. A horizontally elongating, prostrate stem which roots at the nodes.
Striate. With fine, parallel lines or marks.
Trigonous. With three sides or angles.
Valve. One of the outer hulls around the dock seed; one of the persistent and enlarged calyx lobes.

SEDGE FAMILY, CYPERACEAE

The sedge family contains a large number of weeds of minor economic importance, representatives of the genera Carex, Cyperus, Scirpus and Eleocharis whose seeds occur principally in those of pasture and turf grasses, and two major weeds, Cyperus esculentus and C. rotundus.

The seed of a sedge is a hard, one-seeded, indehiscent achene with a thick fruit coat. Usually it is black or dark-brown in color. The inside

of the seed is almost entirely filled with stored food, endosperm, the embryo being extremely small and located at the base of the seed. In the genus Carex, the achene is initially surrounded by a beaked sac-like structure which is frequently persistent on seeds present in agricultural seed. Cyperus seeds strip free of the enveloping bracts and commonly possess a rugose or "alligator-skin-like" outer surface--a characteristic exhibited by both of the noxious species.

NUTGRASS (Cyperus Rotundus L.)
Figs. 1, 2, 3.[1]

Status under law. Prohibited. Alabama, Arizona, Arkansas, Florida, Georgia, Louisiana, Mississippi, Missouri, North Carolina, Oklahoma, South Carolina, Utah, Wyoming (as perennial nutgrass).
Secondary noxious. California (as purple nutgrass), Washington (as perennial nutgrass).

How produced. The achenes are borne in linear, reddish-brown spikelets in the axils of bracts which hide them from view. They shell out of these bracts at maturity.

Size. 1.2-1.5 (1.8) mm. long; 0.6-0.9 mm. wide.

Description. Seeds trigonous with rounded edges, short-oblong to elliptic in longitudinal outline, equally tapering at both ends, short-apiculate at apex. Sides flat or somewhat shrunken and concave. Mature seeds dark-brown, or greyish-brown from a superficial, scurfy bloom; immature seeds sometimes dull grey. Surface cellular-reticulate.

Seeds with which nutgrass may be confused.

YELLOW NUTGRASS (Cyperus esculentus L.). Seeds yellowish to medium brown in color, with or without a scurfy whitish coating, usually broadest above middle and more gradually tapering to base. Cellular reticulations coarser than in C. rotundus (comparison of known samples is necessary to establish this difference in mind), frequently somewhat translucent and bulbous.

Cyperus spp. The seeds of a number of species of Cyperus are said to closely resemble those of the two noxious species, C. esculentus and C. rotundus. Among those which we have examined (about 20 species) we have found none which should be confusing if the analyst is relatively familiar with the two above species. Seeds of C. ovularis (Michx.) Torr. and C. filiculmis Vahl. are somewhat similar to those of C. rotundus and C. esculentus. Seeds of C. ovularis resemble the more oblong seeds of C. esculentus in shape, color and surface texture, but are considerably larger, about 3 mm. in length. The surface markings and color of C. filiculmis are similar to C. rotundus, but the seeds (as long to half again longer than those of rotundus) are more broadly trigonous than the latter, exceeding 1 mm. in width. C. houghtonii Torr. and C. schweinitzii Torr. are similar to C. filiculmis but somewhat larger.

[1] Fig. 1 is an illustration of Cyperus rotundus. Figs. 2 and 3 are of similar seeds with which it is compared. The same procedure is followed throughout the text.

Fig. 1. Nutgrass (Cyperus rotundus). Achenes and enlargement of tip to show reticulation. x 15 and x 36.

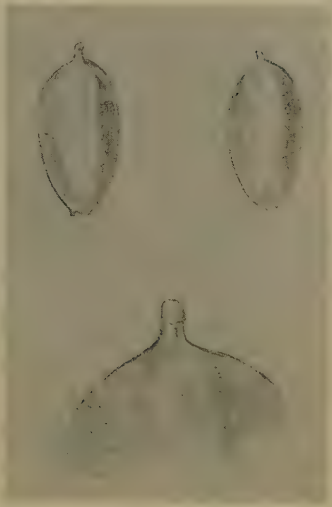

Fig. 2. Yellow Nutgrass (Cyperus esculentus. Achenes and enlargement of tip to show reticulation. x 15 and x 36.

Fig. 3. Cyperus spp. Left to right. Cyperus filiculmis, C. ovularis, C. rotundus, C. esculentus. x 11.

Importance and distribution as a weed. Nutgrass, an introduction from Asia, is a major weed on cultivated soil in the southern and southwestern cottonbelt area of the United States. It also occurs in the Pacific States, the irrigated southwest, and sporadically elsewhere. Nutgrass is perennial from small tubers which are produced in profusion by slender rhizomes. The seeds are frequently low in viability. Justice and Whitehead (1946), studying the production and viability of seeds of this species state, "reproduction by means of seeds is relatively unimportant in the southern

United States".[1] On the other hand, they found C. esculentus to reproduce freely by seeds.

Occurrence in agricultural seed. Rare in southern grown grasses and legumes.

REFERENCES

Bellue (1949), 51-52). Comparative description and illustrations.
Hillman and Henry (1945, pl. 4, Fig. 1). Illustration.
Isely and Wright (1951). Illustration and description.
U.S.D.A. (1952), Figs. 206, 207). Illustrations.
Musil (1942, 53-54; Fig. 36, i.j.). Description and illustrations.

YELLOW NUTGRASS (Cyperus esculentus L.)
Figs. 1, 2, 3.

Status under law. Prohibited. Arizona, Oklahoma, Maine, New Mexico, Wyoming. Secondary noxious. California. Some of these states designate this species as yellow nutgrass and others as nutgrass.

How produced. The achenes are borne in linear, yellowish-brown spikelets.

Size. 1.3-1.8 mm. long; 0.6-0.7 mm. wide.

Description. Seeds (achenes) trigonous with rounded edges, short-oblong to obovate in longitudinal outline, usually abruptly narrowed to an apiculate projection at apex, and more gradually tapering to base. Sides concave or conves. Surface medium- to yellowish-brown, somewhat shiny, cellular-reticulate, the interspaces translucent and somewhat bulbous.

Seeds with which yellow nutgrass may be confused.
See nutgrass (Cyperus rotundus).

Distribution and importance as a weed. Yellow nutgrass occurs throughout most of temperate North America, and also in Europe and Asia. In the United States it is most important as a weed in the northeast and in irrigated crops in the west, especially thriving in low, poorly drained soil. The plant grows as a perennial with slender rhizomes which bear small, nut-like tubers. These latter structures render it difficult to eradicate even after continued cultivation.

Occurrence in agricultural seed. Occasional in small-seeded legumes and grasses.

REFERENCES

(See nutgrass (Cyperus rotundus)).

[1] Dr. Justice has recently informed us, by correspondence, that high germination has been obtained from seeds of C. rotundus from California; seeds of this species from the Anglo-Egyptian Sudan have also been found to possess a high viability. Therefore, in some areas seeds may be an important means of reproduction of this plant.

GRASS FAMILY, GRAMINEAE

The structural appearance of grass seeds is various depending upon the extent to which hulls surrounding the grain are present or have been destroyed during processing. Critical interpretation requires a working knowledge of the relationship between the seeds proper and the accessory protective structures.

The flowering unit, within the grass inflorescence (usually a panicle or spike), is the spikelet. This structure contains one or several flowers surrounded by bracts or hulls. The outermost hulls of the spikelet are the glumes; these are two in number at the base of the spikelet, one on each side (rarely one or both reduced or absent). Above the glumes are florets. In some grasses, several florets are present, alternating on opposite sides of the spikelet axis; in others, only a single floret is present, occupying essentially a terminal position on a short spikelet axis. Each floret contains two hulls, which lie together in clam-shell fashion and enclose a flower. One of the hulls is ordinarily larger than the other and is termed the lemma; the smaller one is the palea. The flower possesses three stamens and a pistil, the latter developing into the grass fruit, grain or caryopsis, and maturing a single seed. Since the fruit does not split open at maturity and shed the seed, the grass seed of commerce is actually a one-seeded fruit, with or without additional accessory hulls.

The nature of the glumes and the number, structure, and positional relationship of the florets is diverse in various grasses and results in corresponding differences in the seeds. The glumes may be long enough to envelop the floret or florets (most frequently the case in one-flowered spikelets), or the florets may be exerted above them. In some cases, one or both glumes are reduced or obsolescent. The glumes may be thin and papery in texture or may be thick and hardened. The lemma and palea are subject to similar variations. In some grasses, sterile florets are present below or above the fertile ones; they are evidenced primarily by the presence of extra lemmas. If located below the seed-bearing florets, these structures sometimes resemble the glumes, if above, they appear similar to the fertile lemmas.

The mode of separation of seed-bearing units from the parent plant is various. The axis of several-flowered spikelets ordinarily breaks between the florets; the "seed" is, then, the matured floret (lemma and palea enclosing a grain) plus a basally attached segment of the spikelet axis, the rachilla. "Seeds" produced by one-flowered spikelets separate either below or above the glumes. In the former case, the "seed" is the entire spikelet consisting of the grain enclosed by an inner hull, lemma and palea, and an outer covering, the glumes plus, in many cases, a sterile lemma. In the latter instance (i.e. the floret disarticulating above the glumes), the seed consists of the matured floret, but without a rachilla.

Examples, including both noxious and nonnoxious genera, of some of the above variations are given below.

Seed a rachilla-bearing, matured floret derived from a several-flowered spikelet: Agropyron, Bromus, Poa, Festuca.

Seed a matured floret, without rachilla, derived from a one-flowered spikelet: Phleum, Agrostis. Immature seeds may still be enclosed within the glumes and thus consist of the entire spikelet.

Seed an entire spikelet with an outer papery hull of glumes (or glume--one of them frequently reduced or obsolescent) and sterile lemma, and a thick hardened inner hull, fertile lemma and palea: Panicum, Digitaria, Setaria, Echinochloa. In the course of processing, the outer or both hulls may be destroyed. In such cases the seed unit is the fertile floret or the hulled grain and is markedly different in appearance than unprocessed seed.

Seed an entire spikelet with a thickened outer hull, glumes, and a tissue paper-like inner hull, lemma and palea: Sorghum.

Seed the hulled grain: Triticum, Secale, Eragrostis (some species). Hulled grains of nearly all grasses may occasionally be found in processed seed.

QUACKGRASS (Agropyron repens (L.) Beauv.)
Figs. 4, 5, 6-16.

Status under law. Prohibited: California, Connecticut, Idaho, Indiana, Iowa, Maine, Massachusetts, Michigan, Montana, New Hampshire, New Jersey, North Carolina, Ohio, Oregon, Pennsylvania, South Dakota, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, Wyoming. Restricted: Canada - (as couch grass), Delaware, Federal Seed Act (import), Florida, Illinois, Louisiana, Minnesota, Mississippi, South Carolina, Tennessee, Oklahoma. Secondary noxious: Colorado, District of Columbia, Kansas, Kentucky, Maryland, Missouri, Nebraska, New York, North Dakota, Rhode Island, Alaska.

How produced. Sessile spikelets are borne in terminal spikes. The spikelets break up at maturity into one-seeded florets. The seed is the rachilla-bearing floret or the dehulled grain.

Size. 6-10 (usually 8-9) mm. long exclusive of awn; about 1.5 mm. wide.

Description. Spikelets 4-6 flowered. Glumes greenish-brown with thick ridge-like nerves, keeled, usually awned.

Lemmas nearly awnless or less frequently awned, usually broadest below middle. Rachilla lying against palea, usually U-shaped at base, of equal width at apex and base, or slightly enlarged towards apex, elliptic in cross section, finely scaberulous. Palea usually glabrous or granular-scabrous, sometimes hairy, not flaring out or markedly flattened at apex; keel bristles stiff, less closely crowded than in other species of Agropyron, (except for crested wheatgrass). Callus broader in the middle than at edges, glabrous or with a few, inconspicuous marginal hairs. A laterally directed hump or bulge is usually present just above the callus--sometimes absent or replaced by a furrow on immature seeds. Hulled grain shaped somewhat like a miniature grain of wheat, brownish in color. Middle portion of germ forming a distinct ridge which is somewhat exerted at base.

Seeds with which quackgrass may be confused.

WESTERN WHEAT GRASS (Agropyron smithii Rydb.). Spikelets 6-10 flowered. Glumes flatter and less strongly nerved than in quack. Seeds, exclusive of awn, 9-10 mm. long. Rachilla lying against palea, usually V-shaped at base, enlarging towards apex, short-hairy (hairs slightly longer than those of quack). Palea usually finely hirsute, especially to-

Fig. 4. Quackgrass (Agropyrons repens). a. Spikelet; b. Mature floret, ventral view; c. Enlargement of apex of floret; d. Enlargement of base of floret and rachilla; e. Base of floret, side view; f. Embryo portion of hulled grain, dorsal and side view; g. Hulled grain. a-b x5. c-g x 10.

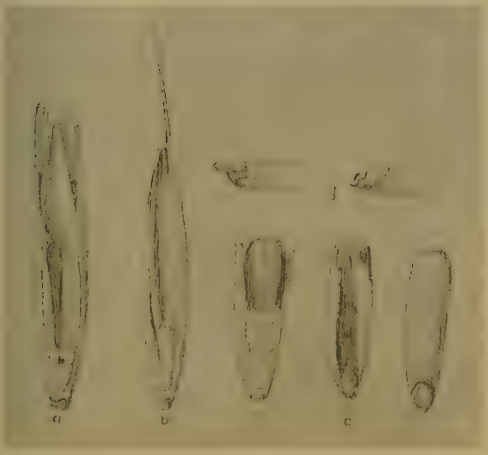

Fig. 5. Quackgrass (Agropyron repens). a. Floret with rachilla slightly enlarged apically; b. Floret with slender rachilla and awn; c. Florets and hulled grain injured or partially hulled during processing; d. Embryo portion of hulled grains, uninjured and injured germ. x7.

wards apex, frequently with a longitudinal furrow near base, the keel teeth somewhat more crowded than those of quack. Callus as in quack, but with marginal hairs more conspicuous (sometimes destroyed in processed seed). A laterally directed furrow rather than a bulge is usually present above the callus. Hulled grain practically indistinguishable from that of quack.

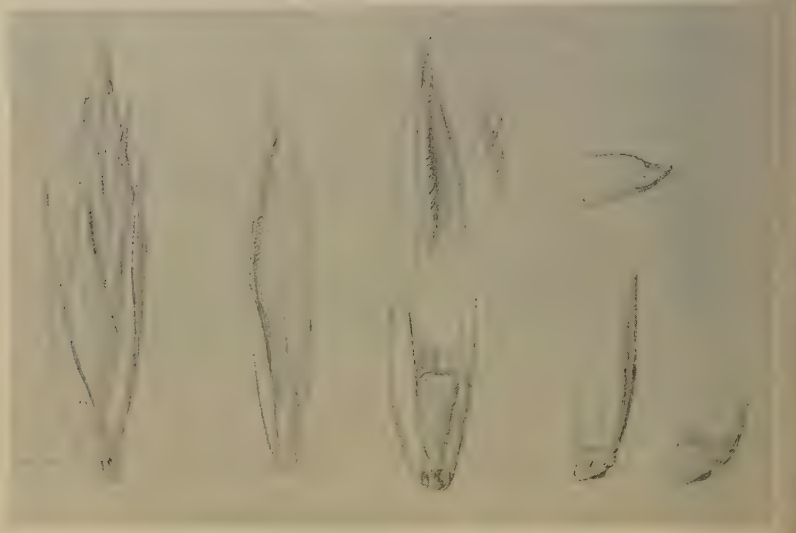

Fig. 6. Western wheatgrass (Agropyron smithii). Spikelet, mature floret and enlargements. Spikelet x3. Floret x7. Enlargement x12.

Of the various incidental seeds similar to quackgrass, western wheatgrass is the most frequently encountered. It is common in brome grass seed, frequently in conjunction with quack.

SLENDER WHEAT GRASS (A. trachycaulum (Link.) Malte). Lemmas usually awnless, widest at or above middle. Rachilla broadening towards apex, silky-hairy, but most of hairs may be destroyed in processed seed. Palea (on well developed seeds) flared out and flattened at apex, the keel teeth finer and more closely crowded than in quack. Callus finely silky-hairy--these hairs frequently subject to partial destruction. Hulled grain thinner, and more broadly flared than that of quack.

CRESTED WHEAT GRASS (A. desertorum (Fisch.) Schult. and also A. cristatum (L.) Gaertn.). Seeds mostly 5-7 mm. long, lanceolate, keeled, with very wide-spaced palea keel bristles.

WILD RYE (Elymus virginicus L.) Spikelets paired; glumes linear-lanceolate, nerveless, bowed at base. Seeds oblong-lanceolate, yellowish, with a straight, scabrous awn. Palea usually exceeding lemma. Base of lemma with lateral furrow, without a bulge.

BLUE WILD RYE (Elymus glaucus Buckl.) Seed flattened especially towards tip. Palea shorter than the gradually tapering lemma. Rachilla

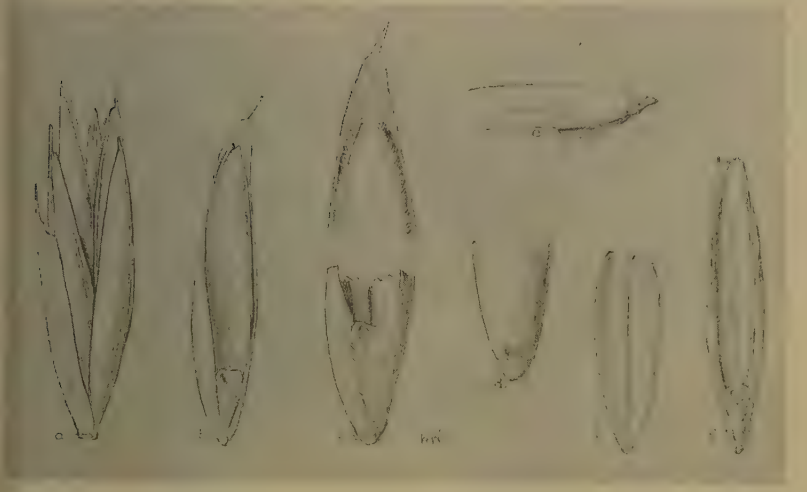

Fig. 7. Slender wheatgrass (Agropyron trachycaulum). a. Spikelet; b. Floret; c, d, e. Enlargements; f. Hulled grain; g. Processed floret--tip of lemma broken and much of pubescence on rachilla destroyed. a-b x 5. c-e, x10. f-g, x5.

Fig. 8. Crested wheatgrass (Agropyron desertorum). Spikelet, mature florets, and enlargements. Spikelet x4. Florets x5. Enlargements x11.

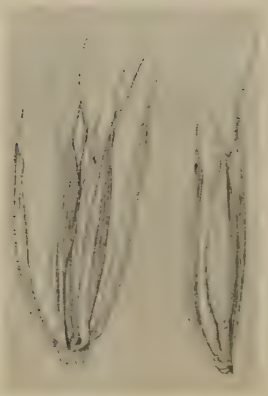

Fig. 9. Wild rye (Elymus virginicus). Left: Node of flowering axis bearing paired spikelets. Right: Single spikelet. x 5.

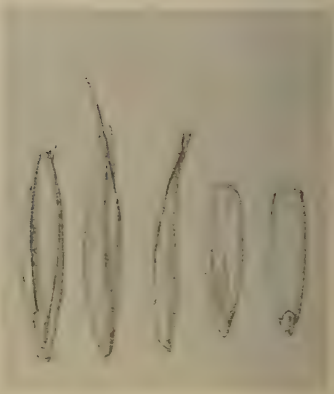

Fig. 10. Wild rye (Elymus virginicus) Mature florets, and hulled grains. x5.

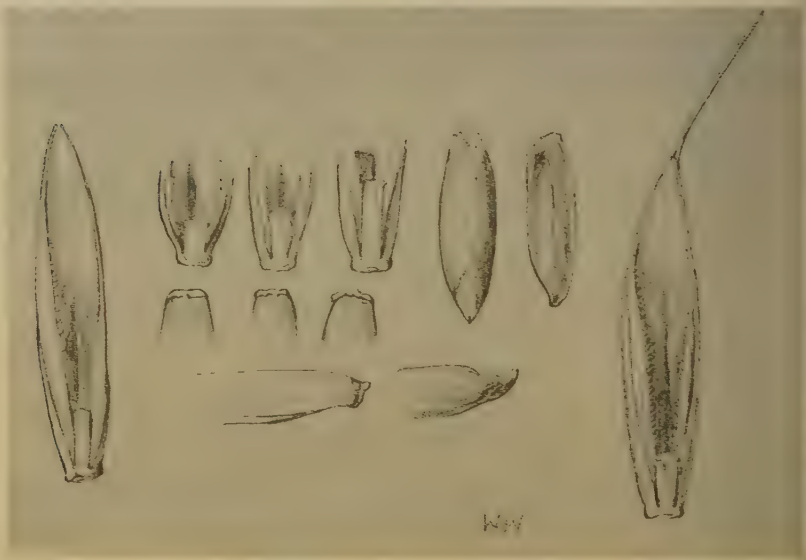

Fig. 11. Ryegrass (Lolium perenne and multiflorum). Mature florets, enlargements, and hulled grains. All L. perenne except L. multiflorum on extreme right. Florets x 7. Enlargements and grains x 15.

slender, usually one-third as long as palea or longer. Base of lemma just above callus irregularly sunken or furrowed. Callus marginally villous (hairs may be destroyed). Occurring primarily in seed from intermountain region.

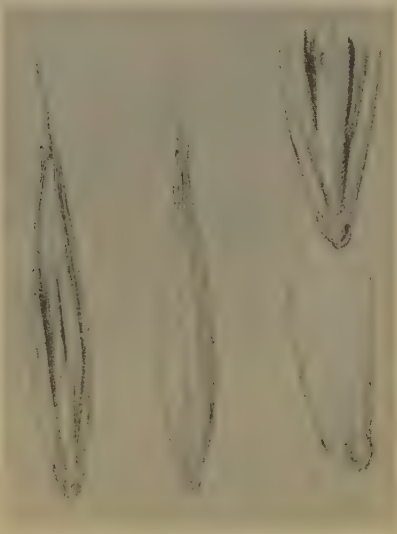

Fig. 12. Blue wild rye (Elymus glaucus). Left and center: Mature florets. Right: Enlargements of base. Florets x7. Enlargements x 15.

RYEGRASS (Lolium perenne L. and L. multiflorum Lam.) Rachilla flattened, narrowly elliptic in cross-section. Callus narrow, of about the same width in middle and at edge. A straight, narrow furrow lies above the callus. The hulled grain is shorter and broader than that of quack; the germ is flatter and with less of a central ridge.

MEADOW FESCUE (Festuca elatior L.) Rachilla usually with an abrupt enlargement at apex. Callus narrow, not broadened in the middle. Grain similar to that of ryegrass. Milled or hulled grains are commonly found in forage grass seed imported from northern Europe.

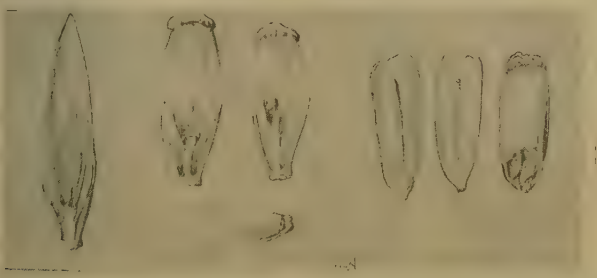

Fig. 13. Meadow fescue (Festuca elatior). Mature floret, enlargements and hulled grains. Florets and grains x 6. Enlargements x 14.

GOATGRASS (*Aegilops cylindrica* Host.) Hulled grain slightly broader and flatter than that of quack and with a distinct brush at apex. Goatgrass is a relatively common weed in Kansas and Oklahoma, but owing to the way in which the seeds are borne (the spikelets partially enclosed within sections of a bony rachis) the hulled grains are not frequently found in agricultural seed.

WHEATGRASSES (*Agropyron* spp.) Several recently introduced Eurasian species of *Agropyron* are now planted to a limited extent for soil conservation, dryland pasture and range renovation purposes. At the present time, the seeds of these plants do not frequently occur as incidental seeds in mixed stocks, this situation may be subject to change in the future.

The seeds of most of these *Agropyron* spp. average somewhat longer than quack seeds and possess a lateral notch at base of lemma; a bulge above this notch may or may not be present. Further specific characters are as follows. *A. intermedium* (Host.) Beauv. Intermediate and Pee wheatgrasses. Edge of lemma extending to palea keels, rachilla not appressed against palea, seeds (excluding awn), 9-10 mm. long. *A. trichophorum* (Link.) Richt. Hairy intermediate wheatgrass: Rachilla and back of lemma villous. *A. elongatum* (Host.) Beauv. Tall wheatgrass: Seeds 10-12 mm. long, appearing conspicuously large and sometimes broad, palea finely pubescent, keel hairs closely crowded; rachilla short, abruptly broadened towards apex. *A. inerme* (Scrib. and Smith) Rybd., Beardless wheatgrass: Seeds 9-10 mm. long. Rachilla gradually enlarged towards apex. Callus somewhat narrowed. Lemma distinctly nerved, usually with 5 veins at apex.

Detection and Diagnosis.

In Oats. The "quack" may occur as entire spikelets or single florets. If the oat sample is put through a blower to remove the chaff, the quackgrass usually goes with the blowings.

In Red or Sweet Clover. Quackgrass seeds may be represented by variously milled florets and hulled grains. The hulled grains are scarcely distinguishable from those of western wheatgrass, and if a sample of red clover contains only a few such grains, a definite identification may be difficult to make. Experience, however, has indicated that such hulled grains are usually those of quack- that hulled western wheatgrass appears to be of rare occurrence in red clover. This assumption can usually be verified if the analyst will obtain a larger seed sample for further examination and find a few seeds with part or all of the lemma and palea remaining.

The germ of hulled grains of quack usually is injured. If more than half of the embryo has been destroyed, the seed may be determined as nonviable.

In Brome. Quackgrass and western wheatgrass are both common in commercial brome seed, and may both be found in the same seed lot; hence, any agropyrons must receive especially careful examination. Florets of quack should also be checked for the presence of a grain inside, as empty, immature florets are of common occurrence.

Distribution and importance as a weed. Quackgrass is of European origin. It now occurs throughout the United States and Canada with the exception of the southern most portion of the United States and is a major

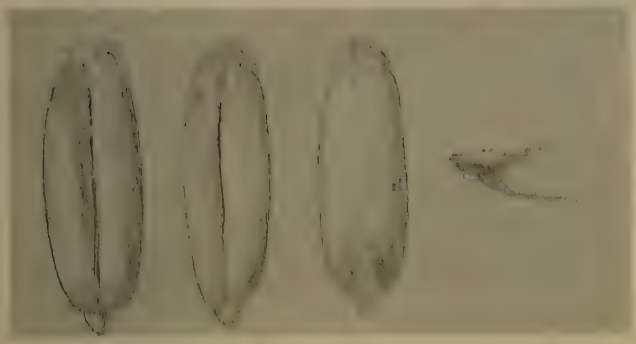

Fig. 14. Goatgrass (Aegilops cylindrica). Hulled grains. x 7.

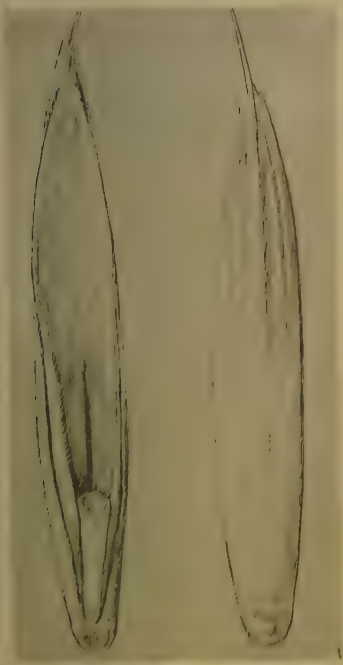

Fig. 15. Beardless wheatgrass (Agropyron inerme). Florets x8.

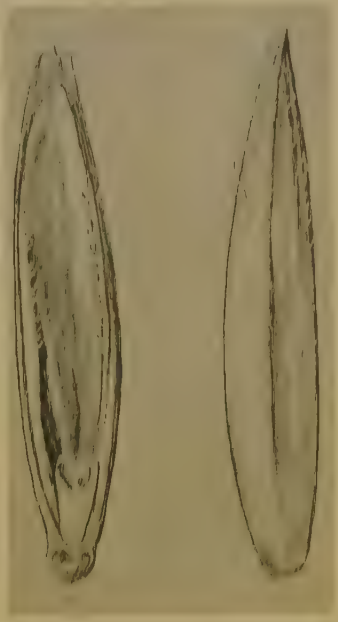

Fig. 16. Hairy intermediate wheatgrass (Agropyron trichophorum). Florets x 8.

noxious weed in the north-central and northeastern states. It is common in cultivated fields, gardens, pastures, legume grass and small grain fields alike. Quackgrass owes its success as a weed to its aggressively spreading rhizome system which will tolerate repeated cultivation.

Occurrence in agricultural seed. Quackgrass seeds are of common occurrence in nearly all kinds of uncleaned agricultural seed produced in the north-central or northeastern states, and adjacent Canadian provinces. Among samples tested in the Iowa State College Seed Laboratory, quackgrass has been most abundant in brome, timothy, red clover, sweet clover and oats. Quackgrass may be found in northwestern seed but is less common; the seeds also occur in importations from northern Europe.

REFERENCES

Bellue (1949, 33). Illustrations and comparative descriptions.
Hillman and Henry (1945, pl. 3, Figs. 12, 19, 20, 22, 23, 24). Illustrations.
Isely (1949, 431-434). Illustrations and comparative descriptions.
Musil (1946). Detailed comparative illustrations and descriptions.
_____ (1948. Descriptions and illustrations of fescue and ryegrass seeds.
_____ (1950). Illustrations and comparative description of all economic species of Agropyron.
U.S.D.A. (1952, 198-201, 214-215, 218, 219-220; Figs. 9-16, 81-84, 93-94, 110). Illustrations and discussion, Agropyron, Lolium, Festuca, and Elymus seeds.
West (1951). Quackgrass and Virginia wild rye.
Wright (1951), Gramineae, Figs. 1, 2, 20). Illustrations, quackgrass and similar seeds.

WILD OATS (Avena fatua L.)
Figs. 17, 18-20.

Status under law. Restricted. Idaho, South Carolina, Utah, Canada. Secondary noxious. Arizona, Colorado, Montana, New Mexico, South Dakota, Texas.

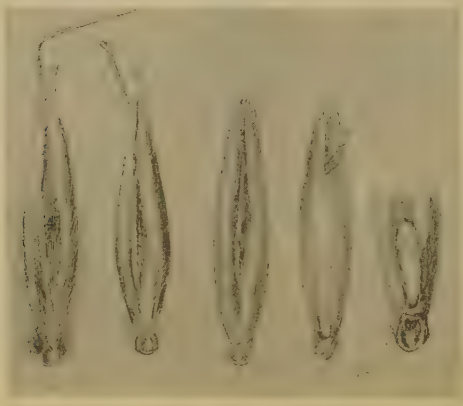

Fig. 17. Wild oats (Avena fatua). Unprocessed seed, processed seed, and enlargement of base. x 4. Enlargement x7.

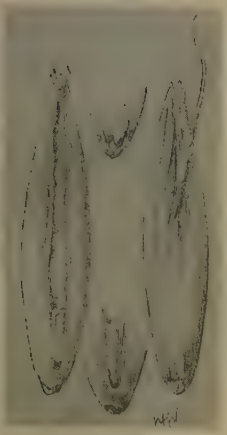

Fig. 18. Cultivated oats (Avena sativa). Florets and slight enlargement of base. x 4.

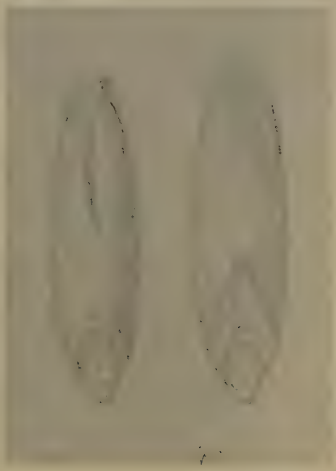

Fig. 19. Hulled grains. Wild oats (Avena fatua), left: Cultivated oats (A. sativa), right. x 4.

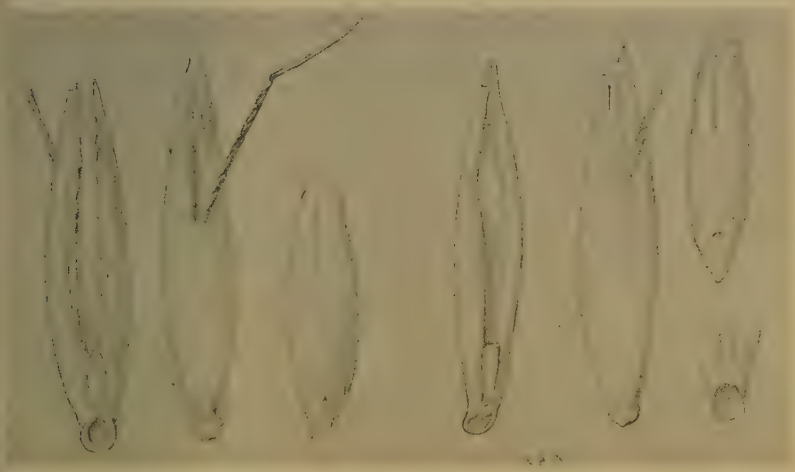

Fig. 20. False wild oats (Avena sativa), Left: Homozygous fatuoid. Right: Heterozygous fatuoid. x 4.

How produced. Two-three-flowered spikelets are borne in a panicle. The florets disarticulate at maturity.

Size. Seed (the floret) exclusive of awn about 1.3-2 cm. long; awn 3-4 cm. in length; hulled grain about 1.0 cm. in length.

Description. Seeds narrowly lanceolate, grey or dark-brown in color, less frequently yellowish to tan. Awn spirally twisted and geniculate, arising from middle of back of lemma, usually partially destroyed on processed seed. Basal attachment scar enlarged into an oblique, conspicuous basal cavity "sucker mouth". Rachilla with a similar oblique scar. Callus, margin of basal cavity, and rachilla with long, stiff hairs which may be mostly destroyed on processed seed. The hulled grain is oat-like in appearance but possesses a groove on the dorsal surface. The scutellum is sunken and its outline is scarcely discernible.

Seeds with which wild oats may be confused.

OATS (Avena sativa L.). Seeds lanceolate (much plumper than those of wild oats), tan to yellow in color, rarely grey, awnless or infrequently awned--the awn not twisted. Basal attachment scar an inconspicuous, jagged break. Callus almost entirely free of hairs. On the hulled grain, the scutellum is superficial and easily discernible externally; the dorsal surface is not grooved.

OATS (Avena byzantina Koch). Seeds as above but reddish-brown in color, frequently awned. Awn straight, geniculate or somewhat twisted. Basal scar inconspicuous, or oblique and somewhat "sucker mouth" like. Callus frequently with inconspicuous tufts of hair.

FALSE WILD OATS (Avena sativa L. and A. byzantina Koch). False wild oats are aberrant forms of cultivated oats. The color and form of the lemma is similar to cultivated oats. Awns present or absent, twisted below or straight. Basal cavity small or conspicuous at base of seed. Margin of basal cavity, callus, and rachilla hairy or glabrate. The scutellum is evident on the hulled grain.

There are two kinds of false wild oats, of which the "homozygous fatuoid" more closely resembles wild oats, differing primarily in size, color, and evidence of the scutellum. Further diagnostic details are given by Musil (1946).

Distribution and importance as a weed. Canada, northern and western United States. Rare in the eastern states but quite common in the northwestern states.

Occurrence in agricultural seed. Wild oats are found primarily in forage grasses and small grain from the northwestern and mountain states and adjacent Canada.

REFERENCES

Hillman and Henry (1945, pl. 2, Fig. 18). Illustration.
Musil (1946a). Seeds of Avena, detailed descriptions and illustrations.
U.S.D.A. (1952, 205-208; Figs. 36-41). Discussion, seed key and illustrations.
Wright (1951 , Gramineae, Fig. 3). Illustration.

CHEAT (Bromus secalinus L.)
Figs. 21, 22-25.

Status under law. Restricted. Alabama, Arkansas, Florida (as Bromus spp.), Georgia, Louisiana, Mississippi, Oklahoma, South Carolina (as Bromus spp.). Secondary noxious. Kansas (as Bromus spp.), New Jersey, New Mexico, Texas.

Most of these states designate this plant by the alternative common names, cheat or chess.

How produced. Several-flowered spikelets are produced in panicles. These spikelets separate into floret segments at maturity and the "seed" represents the matured floret or the hulled grain.

Size. 6.0-7.8 mm. long (exclusive of awn); 1.5-2 mm. wide.

Description. Seeds oblong to lanceolate, about as thick as wide, frequently appearing short and stubby as compared to seeds of other Bromus species. Lemma laterally folded or concave, the margins frequently contiguous and nearly hiding palea from view; surface nearly glabrous; awn rudimentary or 1-6 mm. long (frequently broken off in agricultural seed), straight or abruptly kinked near base. Palea sunken in ventral groove of grain, frequently scarcely discernible, approximating the lemma in length to slightly shorter (-0.3 mm.); keel bristles coarse, widely spaced. Rachilla bowed; apical scar oblique. Grain oblong, convex on dorsal side, deeply grooved ventrally, thick and heavy when mature.

Seeds with which cheat may be confused.

HAIRY CHESS (Bromus commutatus Shrad.). Seeds usually 7.4-8 mm. long (exclusive of awn); appearing thinner and more fragile than mature seeds of cheat; lemma exceeding grain and palea, frequently flared towards apex; awn 5-10 mm. long (commonly destroyed in agricultural seed). Grain much thinner than mature cheat.

Seeds of Bromus commutatus are frequently found in conjunction with those of B. secalinus and are difficult to distinguish from immature seeds of the latter species. The reader is referred to a detailed study of the distinction between seeds of these species (Isely, 1951) in which it is proposed that arbitrary distinctions between doubtful seeds be made on the basis of lemma and palea length. This procedure has since been recommended by the Standardized Test Committee of the Association of Official Seed Analysts (Leggatt, 1951).

JAPANESE BROME (Bromus japonicus Thunb.). Lemma considerably exceeding palea and grain, usually sharply folded; apical scar of rachilla nearly vertical; grain thin; awn commonly bent backwards.

SOFT CHESS (Bromus mollis L.). Lemma finely hair--most of the pubescence may be destroyed on processed seed; lemma and palea usually flat, not keeled or involute. Grain dorsiventrally compressed, thin.

HAIRY CHESS (Bromus racemosus L.). Similar to soft chess but lemma not hairy.

Detection and diagnosis. Cheat in wheat or oats is reasonably easy to detect (although it might be missed by over-hasty analysis). The seeds present are usually mature and well filled and are not likely to be confused with those of other species. In forage grasses, however, small, immature, broken, or "rubbed" seeds are frequently encountered. Such seeds must be carefully examined if confusion with related species is to be avoided.

Fig. 21. Cheat or Chess (Bromus secalinus). Florets and hulled grains. x 6.

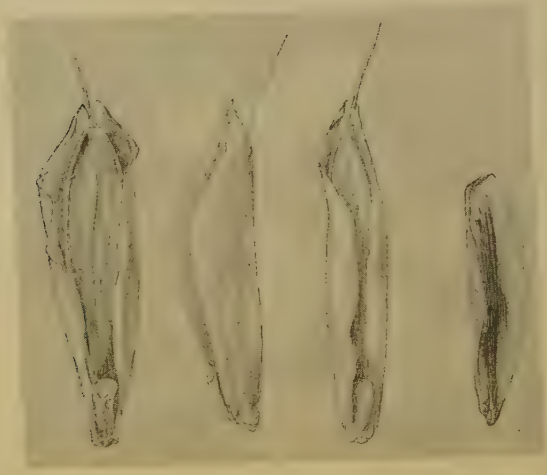

Fig. 22. Hairy chess (Bromus commutatus). Florets and hulled grain. x 6.

Fig. 23. Japanese brome (Bromus japonicus). Florets and hulled grain. Part of lemma cut away in 3rd drawing from left to show palea. x 6.

Fig. 24. Hairy chess (Bromus racemosus). Florets and hulled grain. x 6.

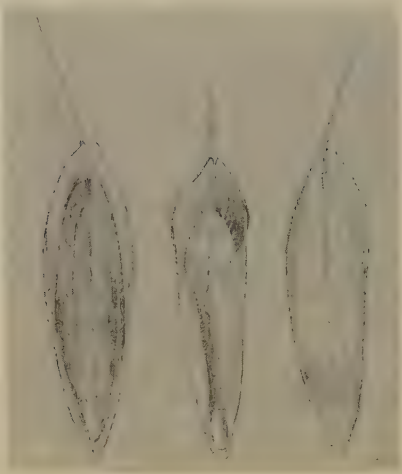

Fig. 25. Soft chess (Bromus mollis). Florets. x 6.

Distribution and importance as a weed. Bromus secalinus is an annual or winter annual introduced from Europe. It is widely distributed throughout the United States and Canada. The plant is a major weed in wheat and, in the southern United States, in oats and forage grasses. In the past, its occurrence in wheat was so consistent that many farmers believed it to represent a "run-down" form of wheat.

Occurrence in agricultural seed. Cheat occurs in forage grasses, especially fescues, ryegrasses and mixtures containing these seeds, in lespedeza and small grains. The seeds are frequently associated with seeds of other bromes especially in forage grasses. The weedy bromes commonly found in orchard grass are usually not cheat.

REFERENCES

Hillman and Henry (1945, pl. 3, Figs. 16, 17, 18). Illustrations. Seed captioned B. racemosus is probably B. commutatus.

Isely (1951). Bromus secalinus and commutatus.

_____ et al. (1951). Seeds of weedy and crop bromes, descriptions and illustrations.

U.S.D.A. (1952, 209-211; Figs. 51-54). Seed key and illustrations.

Wright (1951, Gramineae, Fig. 8). Illustration.

DOWNY BROME (Bromus tectorum L.)
Figs. 21-25, 26, 27.

Status under law. Restricted. Canada. Secondary noxious. Kansas (as Bromus spp.; see heading "Bromus spp." for interpretation).

How produced. Several-flowered spikelets are borne in panicles. The seed consists of the matured rachilla-bearing floret.

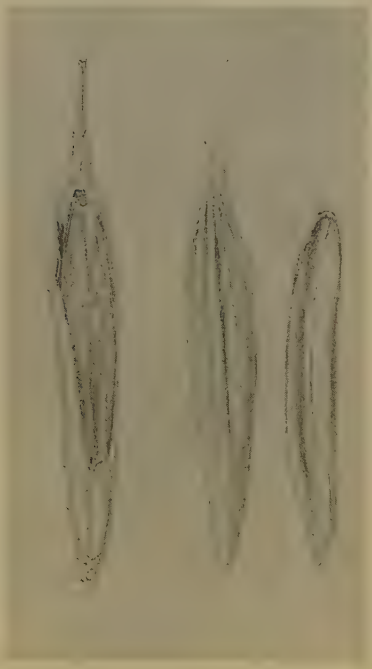

Fig. 26. Downy brome (Bromus tectorum). Florets and hulled grain. x 6.

Size. 7-11 mm. long exclusive of awn.

Description. Floret slender, narrowly oblong, usually curved backwards, long awned. Lemma finely hairy (rarely glabrous), frequently purplish in color, terminating, above attachment of awn, in a pair of membranous teeth, 2-3 mm. long. Rachilla villous with a vertical scar. Palea with a medial ridge or fold; keel bristles capillary, various in length. Grain narrowly oblong, boat-shaped.

Seeds with which downy brome may be confused.

Bromus rubens L. Seed 10-14 mm. long exclusive of awn, nearly flat; palea usually with three longitudinal ridges or folds. Mostly Pacific and southwestern states.

Cheat and related species (B. secalinus L., B. commutatus Shrad., B. mollis L., B. racemosus L., B. japonicus Thunb.). Seeds broader, lanceolate to oblong, straight or backwardly bent; lemma glabrate, or hairy in B. mollis, frequently short-awned; apical teeth inconspicuous, 0.2-1.0 mm. long. Palea keel bristles stiff, nearly uniform in length. Rachilla glabrate or short hairy; scar oblique or vertical.

Distribution and importance as a weed. Downy brome is an annual or winter annual introduced from Europe. It occurs throughout most of the United States and Canada, but is abundant primarily in the western half of the country. It usually occupies noncultivated soils.

Occurrence in agricultural seed. Downy brome seeds are not frequently found in agricultural seed. Among samples tested in the Iowa State College Seed Laboratory it has usually been found in smooth brome and fescues.

REFERENCES

Hillman and Henry (1945, pl. 3, Fig. 15). Illustration.
Isely et al. (1951). Seeds of weedy and crop bromes, descriptions and illustrations.
Korsmo (1953, Fig. 102). Description and illustration.
U.S.D.A. (1952, 209-211; Figs. 49-54). Seed key and illustrations.
Wright (1951, Gramineae, Fig. 9). Illustration.

CHEATS OR CHESSES (Bromus spp.)
Figs. 21-27.

Bromus spp. are restricted in Florida and South Carolina, and are secondary noxious in Kansas.

In regard to species which may be considered noxious under the above regulations, we are informed by correspondence from Florida: "We follow the list as set down by the Seed Control Officials of the Southern States." (Taylor, 1951). Since this list (Association of Seed Control Officials of the Southern States, 1951) specifies only Bromus secalinus, it is inferred that this species is considered noxious in Florida, others excluded. Only Bromus secalinus is considered noxious in South Carolina (Jones, 1951). With reference to the situation in Kansas, Hartley (1951), has written ".....all Bromus with the exception of those considered as crop seed, ... are to be considered noxious weeds .....Bromus secalinus and B. commutatus etc. are the species of borme that are most common in this area".

Exclusive of Bromus secalinus and B. tectorum, previously treated, several, introduced, annual species of Bromus occur in agricultural seed and could be considered noxious under the Kansas interpretation. Seeds of the species enumerated below are briefly described under Bromus secalinus (pp. 541-545).

Hairy chess (Bromus commutatus Shrad.). Hairy chess occurs throughout much of the United States, but is most common as a weed in the northeastern and Pacific states. Its seeds are probably the most abundant of any wild brome in agricultural seed. Seeds of B. commutatus and B. secalinus commonly occur together in forage grasses and must be distinguished seed by seed--see B. secalinus.

Seeds of Bromus commutatus have been widely identified as B. racemosus by analysts in the past.

Japanese brome (Bromus japonicus Thunb.). Japanese brome is most common in the north-central states, largely replacing the hairy chess (B. commutatus) of the eastern states. Its seeds are extremely common in those of the larger-seeded forage grasses, especially commercial brome.

Soft chess (Bromus mollis L.). Soft chess is distributed throughout the United States but is most common in the Pacific states. It is commonly found in ryegrass and orchard grass.

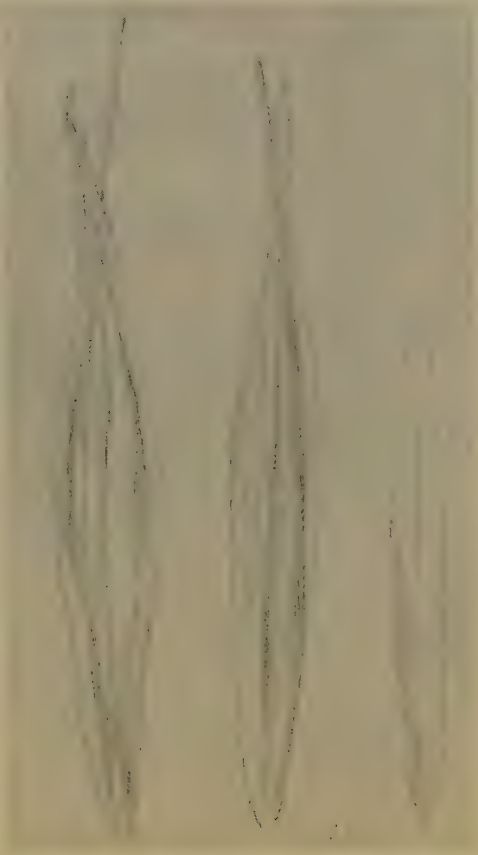

Fig. 27. Bromus rubens. Florets and hulled grain. x 8.

Hairy chess (B. racemosus L.). This species is most frequently encountered in westerngrown forage grasses. Seeds may also be found in grass seed imported from northern Europe.

Bromus arvensis L. This species is of rare occurrence in the United States. Most reports appear to have resulted from confusion with B. japonicus. The seeds are similar to those of B. japonicus but are frequently purplish in hue and possess a palea subequal to the lemma. They are sometimes found in imported seed from Europe.

REFERENCES

Isely et al. (1951). Description and illustrations.
Korsmo (1953, Figs. 78-79, 154-155). Descriptions and illustrations.
U.S.D.A. (1952, 209-211; Figs. 51-54). Seed key and illustrations.

SANDBUR (Cenchrus pauciflorus Benth.)
Fig. 28.

Status under law. Secondary noxious. Arizona (as Burgrass), California, Nevada.

How produced. One-seeded spikelets (usually two) are produced within a spiny bur. The bur, the spikelet, or the hulled grain, may be termed the seed.

Size. Burs 4-6 mm. across, excluding the spines; spines 3-5 mm. long; hulled grains about 3-5 mm. long.

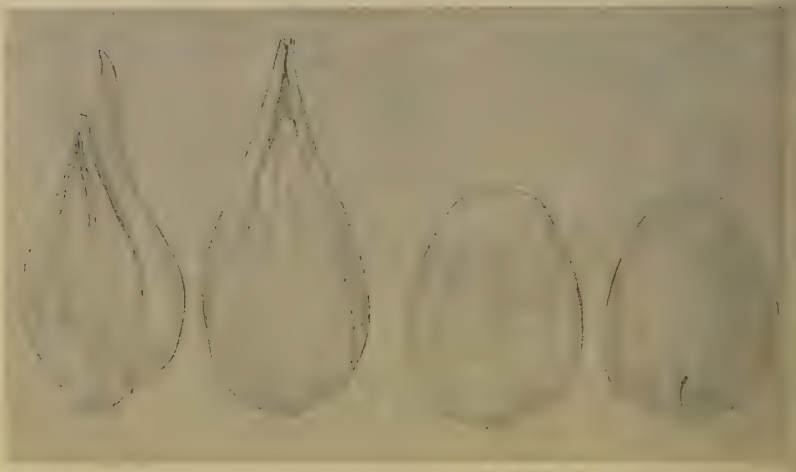

Fig. 28. Sandbur (Cenchrus pauciflorus). Spikelets and hulled grain. x 12.

Description. Bur subglobose, straw colored, with irregularly disposed spines protruding in all directions. Spikelets planoconvex, attenuate-pointed at apex; glumes and lemmas thin and papery. Grain planoconvex, yellow or brownish-red tinged; embryo approaching length of grain, located on flat side; a conspicuous, darkened attachment scar present at base of curved back.

Seeds with which sandbur may be confused.

OTHER SANDBURS (Cenchrus spp.). The burs of Cenchrus pauciflorus are similar in general appearance to those of other representatives of this genus, but since C. pauciflorus is the only species occurring throughout the United States--the other mostly being restricted to the extreme eastern or southern states--the probability of confusion may be remote. Detailed descriptions of the burs are given in Hitchcock and Chase (1950).

DEHULLED GRAINS; Seeds of certain members of the genera Setaria, Echinocloa and Panicum, and small hulled grains of canes or Sudan grass may resemble those of sandbur. However, the embryo of these weed seeds is located on the curved dorsal side of the grain; in sandbur, it is on the flat side.

Distribution and importance as a weed. Sandbur is widespread throughout North America; but is most common in the south. It prefers sandy soil, but will do well under a variety of ecological conditions. It is common in gardens, fields, pastures, and orchards, particularly around human habitations. Forage from young plants is said to be nutritious. The burs on mature plants may be injurious to stock.

Occurrence in agricultural seed. Hulled grains occasionally occur in Sudan, small grains, and legumes. Entire burs are sometimes found in cotton seed.

REFERENCES

Bellue (1949, 59-60). Comparative descriptions and illustrations.
Hillman and Henry (1945, pl. 1, Fig. 20). Illustration.
U.S.D.A. (1952, 211; Fig. 63). Description and illustrations.

BERMUDA GRASS (Cynodon dactylon (L.) Pers.)
Figs. 29, 30.

Status under law. Prohibited. Utah. Restricted. Alabama, Arkansas, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina. Secondary noxious. California, Maryland, New Jersey, Virginia, District of Columbia.

How produced. The one-flowered spikelets are imbricated in spikes. The seed is the matured floret or hulled grain.

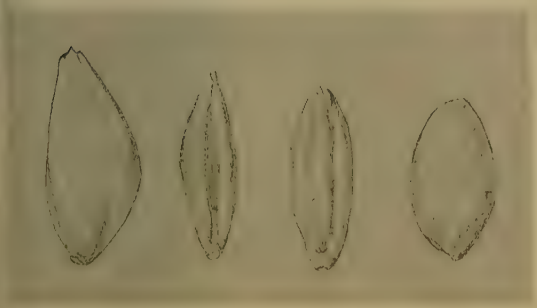

Fig. 29. Bermuda grass (Cynodon dactylon). Florets and hulled grain. x 12.

Size. Florets about 2 mm. long, 1 mm. wide. Hulled grains 1.0-1.2 mm. in length.

Description. Seed laterally compressed, curved on dorsal edge, nearly straight on palea side. Lemma keeled, smooth and coriaceous over entire surface except for line of pubescence along keel. Palea surface narrow. Rachilla inconspicuous. Grain ellipsoidal, laterally com-

pressed, finely striate, frequently with a scurfy or silvery covering. Apex usually with a papillate projection. Embryo one-third to one-half length of grain.

Fig. 30. Hulled grains. Left to right: Bermuda grass (Cynodon dactylon); Annual bluegrass (Poa annua); Sprangletop (Leptochloa dubia); Dropseed (sporobolus cryptandrus). x 12.

Seeds with which Bermuda grass may be confused.

ANNUAL BLUEGRASS (Poa annua L.). Hulled grain: Surface of grain finely bumpy, not silvery, scarcely striate. Embryo one-fourth to one-third length of grain.

SPRANGLETOP (Leptochloa dubia (H.B.K.) Nees.). Hulled grain: grain dorsally compressed.

DROPSEED (Sporobolus cryptandrus (Torr.) Gray, and other species). Hulled grain: Grain not silvery, often somewhat opaque, scarcely striate, usually finely cancellate; embryo frequently darker than grain.

Distribution and importance as a weed. Bermuda grass, a native of India, is abundant throughout the southern half of the United States both as a crop and as a weed. It spreads aggressively by both rhizomes and stolons. Bermuda grass thrives in various soils and habitats, in both cultivated and noncultivated areas. It is tolerant of drought.

Occurrence in agricultural seed. Bermuda grass seed may be found in nearly any kind of southern grown legume or grass seed.

REFERENCES

Bellue (1949, 43-44). Comparative illustrations and descriptions.
Hillman and Henry (1945, pl. 2, Figs. 4, 21). Illustrations.
U.S.D.A. (1952, Figs. 62, 197). Illustrations.
Wright (1951, Gramineae, Fig. 12). Illustrations.

SMOOTH CRABGRASS (Digitaria ischaemum (Schreb.) Muhl.) Figs. 31, 32.

Status under law. Restricted. Canada (as Digitaria spp. in turf grasses only). Secondary noxious. Nevada, New York (as Digitaria spp.), Rhode Island (as Digitaria spp.).

How produced. One-flowered spikelets are borne in digitate spikes. The seed may represent the entire spikelet, the mature floret, or the hulled grain.

Size. 2 mm. long, 1-5 mm. wide (spikelet).

Description. Spikelets plano-convex, ovate, pointed. Outer hulls (second glume and sterile lemma) papery, finely hairy; second glume about equaling the seed in length. Inner hulls (fertile lemma and palea) thick and hardened, black when mature, longitudinally striate with rows of fine granules. Grain whitish to yellow-white, plano-convex, broadly oblong; embryo 1/3-1/2 length of grain.

Seeds with which smooth crabgrass may be confused.

CRABGRASS (Digitaria sanguinalis (L.) Scop.). Seeds about 3 mm. long, ovate-lanceolate. Second glume (hairy outer hull on the back) about half the length of seed. Fertile lemma (hard inner hull) dull yellow, olive-gree or brownish depending on stage of maturity.

OTHER CRABGRASSES (Digitaria spp.). Certain other crabgrasses, the seeds of some of which resemble those of smooth crabgrass, occur within the United States but are primarily restricted to the southeastern coastal plain, especially Florida. Their seeds rarely occur in agricultural seed.

Distribution and importance as a weed. Smooth crabgrass is a European introduced annual which is now common in eastern North America. It may be found on both cultivated and noncultivated soil. Through its ability to form numerous, prostrate tillers which root at the nodes, it is of especial importance as a late summer lawn weed.

Occurrence in agricultural seed. Seeds of smooth crabgrass occur incidentally in seed lots of nearly any small-seeded legume or grass, or mixture.

Fig. 31. Smooth crabgrass (Digitaria ischaemum). Spikelets, mature floret (dorsal view), and hulled grains. x 15.

REFERENCES

Hillman and Henry (1945, pl. 1, Figs. 6, 7). Illustrations.
U.S.D.A. (1952, Figs. 73-75). Illustrations.

CRABGRASS (Digitaria sanguinalis (L.) Scop.)
Figs. 31, 32, 33.

Status under law. Restricted. Canada (as Digitaria spp.; lawn and turf grasses only). Secondary noxious. Nevada, New York (as Digitaria spp.), New Hampshire, Rhode Island (as Digitaria spp.).

How produced. Same as smooth crabgrass.

Size. 3 mm. long, 1-1.5 mm. wide (spikelet).

Description. (See smooth crabgrass). Outer hulls (second glume and sterile lemma) hairy; second glume about half length of seed. Fertile lemma (hard inner hull) dull yellow, olive-green to brown depending upon maturity, rough granular under magnification.

Seeds with which crabgrass may be confused.

SMOOTH CRABGRASS (Digitaria ischaemum (Schreb.) Muhl.). (See comparative descriptions under smooth crabgrass).

FALL WITCHGRASS (Leptoloma cognatum (Schult.) Chase). May be confused with crabgrass if outer hulls are absent. Seed about 2.5 mm. long, 0.7-1.0 mm. wide. Fertile lemma yellow to brown (no olive tinge), finely granular or with broken longitudinal ridges under high magnification (comparison of known seeds is necessary to establish this difference).

Distribution and importance as a weed. Same as smooth crabgrass.

Occurrence in agricultural seed. Same as smooth crabgrass.

Fig. 32. Crabgrass (Digitaria sanguinalis). Spikelets, mature floret (dorsal view), and hulled grains. x 15.

REFERENCES

Hillman and Henry (1945, pl. 1, Figs. 6, 7). Illustrations.
Musil (1944). Description and illustration of Leptoloma cognatum.
U.S.D.A. (1952, Figs. 73-75, 109). Illustrations.
Wright (1951, Gramineae, Fig. 16). Illustration.

Fig. 33. Fall witchgrass (Leptoloma cognatum). Spikelet, mature florets. x 15.

CRABGRASSES (Digitaria spp.)
Figs. 31, 32

The Canadian, New York, and Rhode Island seed laws specify Digitaria spp. as noxious; the former restricts the sale of certain turf grasses and lawn mixtures containing these seeds; the latter two treat Digitaria seeds as secondary noxious.

We have interpreted Digitaria spp. to include Digitaria sanguinalis, and D. ischaemum, the two wide-spread weedy members of this genus.

SQUIRREL-TAIL GRASS (Hordeum jubatum L.)
Figs. 34, 35, 36, 37.

Status under law. Secondary noxious. Arizona.

How produced. Inflorescence a dense spike bearing three spikelets to a node. At maturity the axis (of the spike) breaks into segments, each consisting of a short section of the rachis and a cluster of the long-awned spikelets. The lateral spikelets are reduced to a pair of bristle-like glumes and a small, long-awned lemma. The central spikelet is one-flowered and fertile; the glumes are represented by a pair of long bristles offset to the back of the floret. The term seed, as popularly applied to wild species of Hordeum, sometimes refers to the unit cluster of spikelets and sometimes to the seed-bearing fertile floret. The description below pertains to the floret.

Size. Length (exclusive of awn) 5-6 mm., width 1.5 mm; awn 20-30 mm. long.

Description. Seed (floret) lanceolate, long-awned. Rachilla represented by a short bristle in front of palea. Lemma convex, slightly bulged above callus; palea bearly flat, with 2-3 longitudinal, medial folds or wrinkles, without marginal keel teeth. Seed glabrous, or with fine hairs under high magnification.

Fig. 34. Hordeum spp. Spikelet clusters showing fertile spikelet and lateral sterile spikelets. Left to right: Squirrel-tail grass (H. jubatum); Wild barley (H. leporinum); Little barley (H. pusillum). x 7.

Seeds with which squirrel-tail grass may be confused.

LITTLE BARLEY (Hordeum pusillum L.). Lemmas and glumes short-awned (8-15 mm.). Glumes of fertile spikelet not reduced to a bristle. Mature florets broader and plumper than in squirrel-tail grass. Palea with distinct sub-marginal keels and an inconspicuous medial ridge. Most common in southern and western United States.

WILD BARLEY (Hordeum leporinum Link.). See description (p. 555).

Distribution and importance as a weed. Squirrel-tail grass occurs throughout the United States with the exception of the southeastern portion. It is widely distributed in Canada.

Occurrence in agricultural seed. The seeds of squirrel-tail grass are occasionally found in those of brome and fescues.

REFERENCES

Isely and Wright (1951). Comparative descriptions and illustrations. U.S.D.A. (1952, 218-219; Figs. 105-108). Seed key and illustrations. Wright (1950, Gramineae, Fig. 25). Illustration.

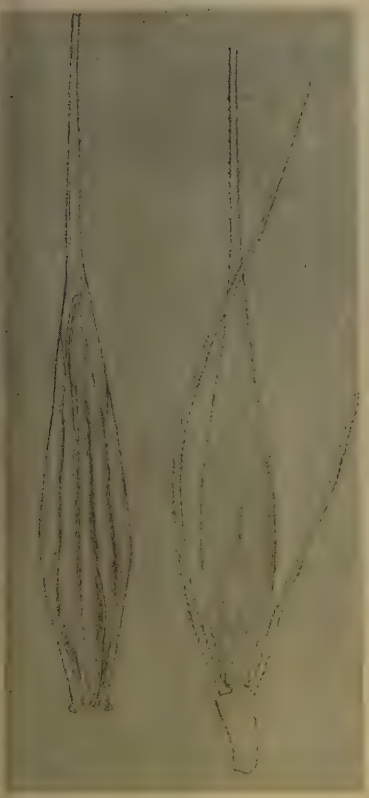

Fig. 35. Hordeum jubatum. Left: fertile floret, palea view; right: fertile spikelet with glumes. x 11.

Fig. 36. Hordeum pusillum Left: fertile floret, palea view; right: fertile spikelet with glumes. x 11.

WILD BARLEY (Hordeum leporinum Link.)
Figs. 34-36, 37.

Status under law. Secondary noxious. Arizona (as H. murinum L.).

How produced. Same as squirrel-tail grass.

Size. 8-10 mm. long, 1.5-2.0 mm. wide; awn 20-30 mm. long.

Description. Similar to squirrel-tail grass, but larger and flatter, the palea face usually deeply grooved or furrowed. Glumes of fertile spikelet ciliate with villous hairs.

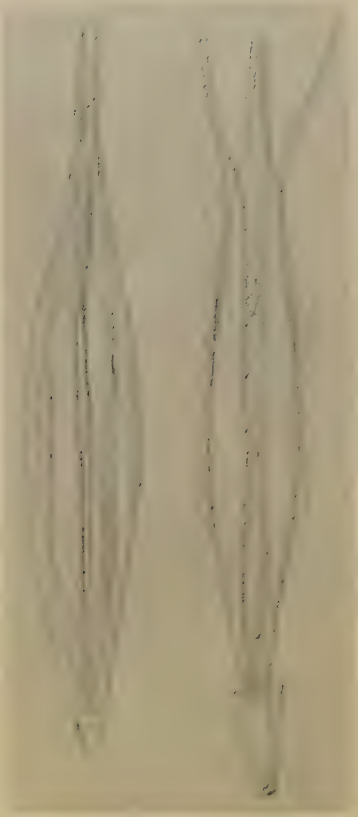

Fig. 37. Hordeum leporinum. Left: fertile floret, palea view; right: fertil spikelet with glumes. x 11.

Seeds with which wild barley may be confused.

SQUIRREL-TAIL GRASS (Hordeum jubatum L.). See description above.

WILD BARLEY (Hordeum pusillum Nutt.). Lemmas and glumes short-awned. Glumes of fertile floret not villous-ciliate. Mature florets shorter and plumper.

Distribution and importance as a weed. Wild barley is common in the western United States and British Columbia; it occurs sporadically in the eastern states and elsewhere.

Occurrence in agricultural seed. We have never encountered the seeds of this weed in agricultural seed lots.

REFERENCES

Isely and Wright (1951). Illustrations and comparative descriptions. U.S.D.A. (1952, 218-219; Figs. 105-108).

Fig. 38. Lolium persicum
Mature florets. x 7.

PERSIAN RYEGRASS (Lolium persicum Boiss. and Hohen.)
Fig. 38.

Lolium spp. other than L. perenne and L. multiflorum are restricted under the Canadian Seeds Act.

L. persicum, an introduced Eurasian plant, is local, primarily as a weed in wheat, in Alberta, Ontario, and North Dakota. Its seeds are contrasted with those of L. temulentum under the latter. In shape it is somewhat similar to common ryegrass but is considerably larger (8-10 mm. long(. Musil (1944) comments that when found in crop seed, the palea and lemma are frequently broken at the end so that the tip of the grain is exposed.

REFERENCES

Musil (1944). Illustration and description (as L. rigidum var. duthiei).
_____ (1948). Illustration and description.
U.S.D.A. (1952; 219-220; Figs. 110-113). Seed key and illustrations.

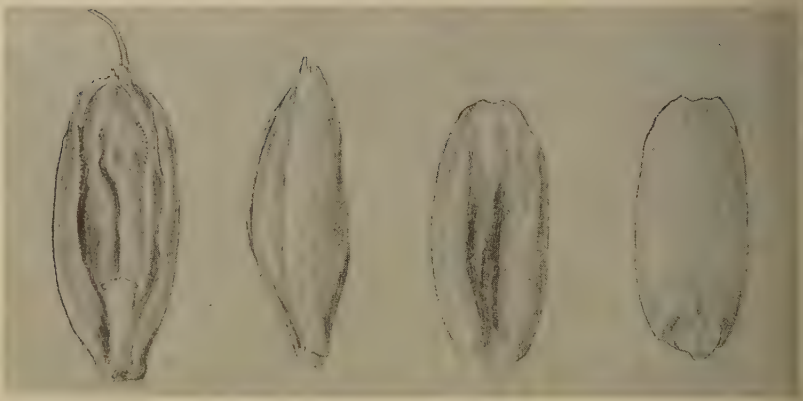

Fig. 39. Darnel (Lolium temulentum). Mature florets and hulled grains. x 6.

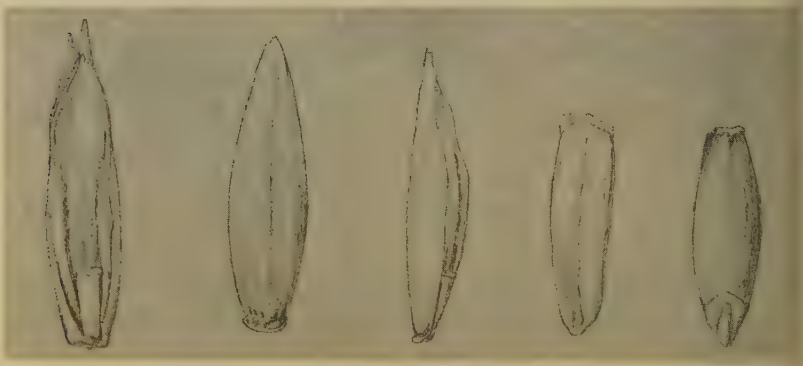

Fig. 40. Ryegrass (Lolium perenne). Mature florets and hulled grain. x 6.

DARNEL (Lolium temulentum L.)
Figs. 21, 38, 39, 40.

Status under law. Restricted. Alabama, Arkansas, Florida, Georgia, Mississippi, South Carolina, Oklahoma, Canada (as Lolium spp. other than L. perenne and L. multiflorum). Secondary noxious. Texas.

How produced. 5-7 flowered spikelets are borne alternately in a terminal spike. The seed is the matured floret with rachilla.

Size. (4) 6-8 mm. long (exclusive of awn), 1.5-3 mm. wide; awn 10-15 mm. in length.

Description. Seeds glabrous, elliptic or broadly oblong, stubby and

plump, when mature nearly as thick as wide. Lemma convex, awned (but awn generally destroyed on processed seed); callus very narrow. Palea, dependent upon maturity of grain, convex, concave or medially furrowed; keels finely ciliate, frequently conspicuously raised above central portion. Rachilla appressed to palea, laterally compressed; elliptic rachilla scar horizontal or slightly oblique. Grain brownish, broadly elliptic in outline, plump when mature, dorsally convex, ventrally grooved; embryo nearly circular.

Seeds with which darnel may be confused.

RYE GRASS (Lolium perenne L. or L. multiflorum Lam.). Seeds oblong-lanceolate, not plump, much thinner than wide, usually not exceeding 2 mm. in width.

CHEAT (Bromus secalinus L.). Palea keel with long bristles. Rachilla frequently bowed away from palea, the apical scar oblique.

PERSIAN RYEGRASS (Lolium persicum Boiss. and Hohen.). Seeds oblong to narrowly oblong, 8-10 mm. in length, thinner than wide. Palea not possessing a medial furrow.

Detection and diagnosis. Small oat seeds are of approximately the same size and color as those of darnel. In the analysis of oats from the southeastern states, it is possible that darnel may be overlooked if too hasty examination is made. Also, since cheat and darnel frequently occur together in such seed, care must be employed that they be distinguished.

Distribution and importance as a weed. Darnel occurs locally throughout the eastern half of the United States and on the Pacific coast; it is common in the southeastern states. The plant is most frequently found in small grains and on noncultivated waste areas.

Darnel has been said to be poisonous--hence the alternative common name, poison darnel. Evidence concerning the presence or nature of the poisonous characteristic is, however, conflicting.

Occurrence in agricultural seed. Darnel is most frequent in small grain seed from the southeastern United States.

REFERENCES

Hillman and Henry (1945, pl. 3, Figs. 19-21). Illustrations of ryegrass and darnel.
Musil (1948). Description and illustrations of darnel and similar seeds.
U.S.D.A. (1952, 219-220; Figs. 110-113). Seed key and illustrations.
Wright (1950, Gramineae, Fig. 26). Illustration.

RED RICE (Oryza sativa L.)
Fig. 41.

Status under law. Restricted. Alabama, Arkansas (in rice only), Florida, Louisiana, Mississippi, South Carolina.

How produced. One-flowered spikelets are borne in panicles. The seed is represented by the entire spikelet or the hulled grain.

Size. Unhulled seeds 8.0-10.0 mm., hulled grains 6.0-7.0 mm. long.

Description. Unhulled seeds (spikelets), body of seed oblong, somewhat laterally flattened, frequently with a stiff awn. A pair of short scale-

like glumes are present at base of seed. Hulls (lemma and palea) stiff and hard, granular-roughened, with short, stiff hairs.

Hulled grains. Grains oblong, laterally compressed, longitudinally furrowed, reddish in color. Embryo on narrow edge, about one-fourth length of grain.

Seeds with which red rice may be confused.

CULTIVATED RICE (Oryza sativa L.). Spikelets indistinguishable from those of red rice but shattering less easily. Grains light (off-white) in color.

Detection and diagnosis. Since red rice in the hull is indistinquishable from ordinary rice, special techniques must be used to determine its presence. The so-called rub-out test may be made by passing rice through a mill or across a rub-out board (described by Randall, 1950). Either procedure serves to remove or break the glumes so that the grain can be observed.

Fig. 41. Rice (Oryza sativa). Left: cultivated rice. spikelet and hulled grain. Right: red rice, hulled grain. x 7.

Distribution and importance as a weed.[1] Red rice is a genetic strain of rice which differs in color of the grain, ease of shattering and earliness. It grows exclusively in rice fields or in soil in which rice enters into the rotation. According to Randall (1950) it "is the most universal and persistent problem found in rice growing throughout the world".

Red rice crosses with commercial rice and may thus increase in seed stocks if an effort is not made to keep them pure. Red rice competes with rice in the field and does not markedly contribute to the yield since the grains are usually lost by early shattering. The commercial product is subjected to severe dockage if red rice is present. Records from California during the last 10 year period indicate an upward trend in proportion of rice seed lots containing red rice (Randall, 1950). The only known control is use of clean seed in noninfested soil (seeds are capable of living 2-3 years in soil).

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[1] Dr. Clair Brown, Professor of Botany, Louisiana State College, contributed information concerning red rice on which these remarks, in part, are based.

Occurrence in agricultural seed. Red rice is found almost exclusively n rice seed.

REFERENCES

Randall (1950). Occurrence in California seed rice.
J.S.D.A. (1952, Fig. 117). Illustration.

PANIC GRASS (Panicum spp.)
Figs. 42,43.

Species under consideration. Seeds of witchgrass (Panicum capillare L.) fall panicum (P. dichotomiflorum Michx.), and switchgrass (P. virgatum L.) are most common in agricultural seed. The descriptions below have primary reference to these species. Seeds of other panicums are, in general, similar to those described.

Status under law. Restricted. Canada (in seeds of lawn and turf grasses only).

How produced. One-seeded spikelets are borne in panicles. The spikelet contains a pair of glumes, sterile lemma, fertile lemma and palea. As found in agricultural seed, the seed is represented by the entire spikelet, the fertile floret, or the hulled grain.

Size. Spikelets 2.5-3.0 mm., florets 1.5-2.0 mm. long in Panicum capillare and P. dichotomiflorum; 3.5-4.5 and 3.0 mm. long in P. virgatum.

Description. Spikelets ovate to ovate-lanceolate, acute to acuminate-pointed. Outer hull (glume or glumes and sterile lemma), papery, longitudinally nerved; inner hull (fertile lemma and palea) hard, smooth and shiny, yellowish (immature) or dark with light longitddinal lines. Floret somewhat plano-convex, pointed. Grain plano-convex, ovate in outline, yellowish to grey-yellowish; embryo about one-third length of grain.

Seeds with which panic grasses may be confused.

Seeds of the common species of Panicum as characterized above should not be subject to confusion with seeds of other grasses. The hulled grains approximate those of several crops in size (e.g. timothy, bluegrass) and may escape detection. If examined, they can easily be distinguished on the basis of shape.

Distribution and importance as weeds. Panicum capillare and P. dichotomiflorum are common in the eastern United States and Canada. Both are rapid-growing, late summer annuals of tilled or uncultivated soil. P. virgatum is a rank perennial, usually found in wet soil. It also is most characteristic of eastern North America, and is generally found along streams, in low, wet pastures or hayfields.

Occurrence in agricultural seed. Seeds of Panicum capillare and P. dichotomiflorum are common contaminants of seeds of nearly all central or eastern produced small-seeded legumes and grasses. Seeds of P. virgatum are less frequently encountered, usually being restricted to seeds of forage grasses.

REFERENCES

Hillman and Henry (1945, pl. 1, Figs. 8, 9). Illustrations.
Musil (1944a). Illustrations and descriptions.
U.S.D.A. (1952, 220-222; Figs. 121-138). Seed key and illustrations.

WIRE GRASS (Paspalum distichum L.)
Figs. 42, 43, 44, 45.

Status under law. Secondary noxious. Texas.

How produced. One-flowered spikelets are borne in spikes. Processed seeds may consist of entire spikelets or, if outer hulls (second glume and sterile lemma) are destroyed, fertile florets.

Size. 3-4 mm. long, 1.5 mm. wide.

Description. Seed plano-convex, ovate in outline, truncate at base. First glume usually absent; the second glume is inconspicuously hairy, particularly towards tip. Fertile floret slightly exceeded by the papery second glume and sterile lemma. Fertile lemma and palea coriaceous, yellowish, faintly longitudinally striate.

Seeds with which wire grass may be confused.

PASPALUMS (Paspalum spp.). Seeds usually wider and rounded at apex. P. laeve Michx., the most common species in southern grown seed, has nearly circular seeds. Seeds mostly glabrous, but if pubescent, strongly so as in Dallis grass (P. dilatatum).

PANICUMS (Panicum spp.). Seeds usually smaller than those of wire grass and with a short first glume. Inner hull (fertile lemma and palea) of common species shiny with 2-6 longitudinal lines.

Distribution and importance as a weed. Extreme eastern, southern and western United States; absent from the major portion of the central and northern states.

Occurrence in agricultural seed. Rare in southern grown grasses and legumes.

REFERENCES

Hillman and Henry (1945, pl. 1, Figs. 3-5, 8, 9). Illustrations of seeds similar to Paspalum distichum.
U.S.D.A. (1952, 222-223; Figs. 139-148). Seed key and illustrations.

JOHNSON GRASS (Sorghum halepense (L.) Pers.)
Figs. 46, 47, 48.

Status under law. Prohibited. Arizona (in Sudan grass and small-seeded legumes), Indiana, Missouri, North Carolina, Ohio, Tennessee, Utah, Virginia, Washington, West Virginia. Restricted. Alabama, Arkansas, Florida, Georgia, Illinois, Kansas, Louisiana, Mississippi, Oklahoma, Oregon, South Carolina, Texas, Canada.
Secondary noxious. Arizona (in crops except Sudan grass and small-seeded legumes), California, Kentucky, Maryland, New Mexico, Alaska.

Fig. 42. Witchgrass (Panicum capillare). Spikelets and florets. x 11.

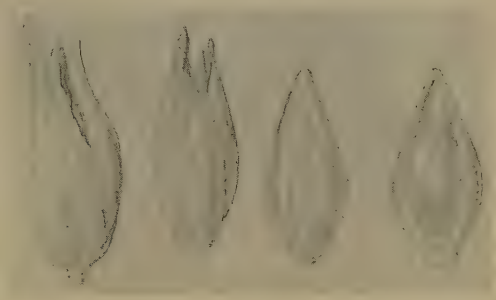

Fig. 43. Switchgrass (Panicum virgatum). Spikelets and florets. x 10.

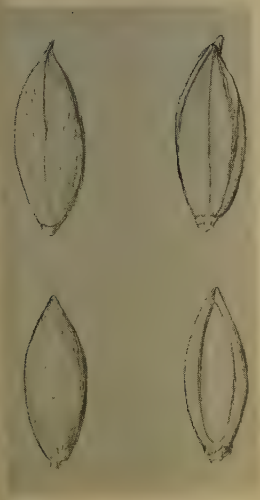

Fig. 44. Wiregrass (Paspalum distichum) Spikelets and florets. x 10.

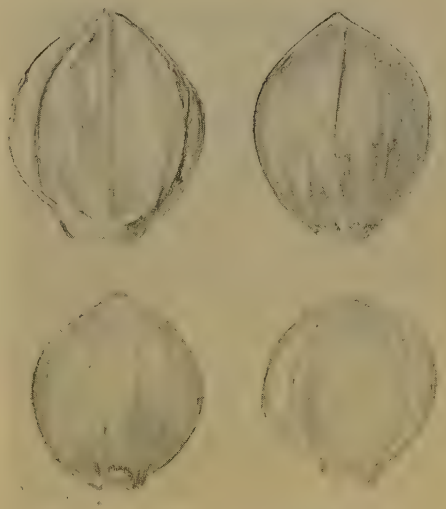

Fig. 45. Paspalum (Paspalum laeve). Spikelets and fertile florets. x 10.

How produced. Inflorescence a panicle. Spikelets paired, one (fertile and seed-bearing) sessile, the other (sterile) stalked. The seed is the one-seeded fertile spikelet.

Size. Unhulled seed: 4-4.5 mm. long, 1.5-2.0 mm. wide. Hulled grain about 2.5-3.0 mm. long. 1.5-2.0 mm. wide, less than twice as long as wide.

Description. Unhulled seeds elliptic at apex, tapering or rounded at base, slightly dorsally compressed, awned, (but awns rarely seen in processed seed). A pair of rachilla-like stalks, the "pedicels" are attached at base of seed.[1] One or both of the pedicels terminate at apex in an enlarged cup-like scar. Glumes (the outer hulls) black to reddish-mahogany at maturity; immature seeds may be partially or completely straw-colored. Grain dark red-brown in color, somewhat glossy, obovoid. Tissue from the attachment scar protrudes downwards slightly beyond main body of seed and approximates tip of radicle, separated from the latter by an indentation (Fig. 46).[2]

Seeds with which Johnson grass may be confused.

SUDAN GRASS (Sorghum sudanense (Piper) Stapf.). Seeds 4.5-5.5 mm. long, pointed at both ends, the majority straw or sienna colored, but some are partially or completely black. Pedicels not terminating in enlarged cup-like scars, but breaking irregularly at apex. Grain elliptic, 3-4 mm. long. Attachment scar tissue not protruding beyond main body of grain, neither reaching tip of radicle nor separated from it by an indentation.

INDIAN GRASS (Sorghastrum nutans (L.) Nash.). Hulled seed 2.2-3.5 mm. long, 1.0-1.6 mm. wide, more than twice as long as wide, usually lighter in color than Johnson grass and tending to be finally striate.

SORGHUM (Sorghum vulgare Pers.). Seeds much larger than those of Johnson grass; pedicels usually not present.

Detection and diagnosis. Since Sudan grass and Johnson grass are superficially very similar, care must be taken in noxious weed examinations of Sudan in regard to the detection of Johnson grass.

Distribution and importance as a weed. Johnson grass, native to the Mediterranean region, was introduced into the southern United States as a forage about 100 years ago. It is now widespread throughout the southern half of the country.

Although other weeds may present more acute local problems, Johnson grass is possibly the most important weed in the southern United States as a whole. It is persistent from rapidly growing rhizomes which give rise to numerous new stems. These stems are lush and leafy, and frequently reach a height of 4-5 feet. The plant is aggressively competitive and, if undisturbed, is capable of completely suppressing most crop plants.

Occurrence in agricultural seed lots. Relatively common in larger-seeded, southern grown legumes and forage grasses. Occasional in cereals.

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[1] These represent: (1) pedicel of sterile spikelet, (2) common stalk of next pair of spikelets.

[2] Observations on this character, which serves to contrast hulled grains of Johnson grass with those of Sudan, were first reported to the writer by Dale West of the Iowa State College Seed Laboratory.

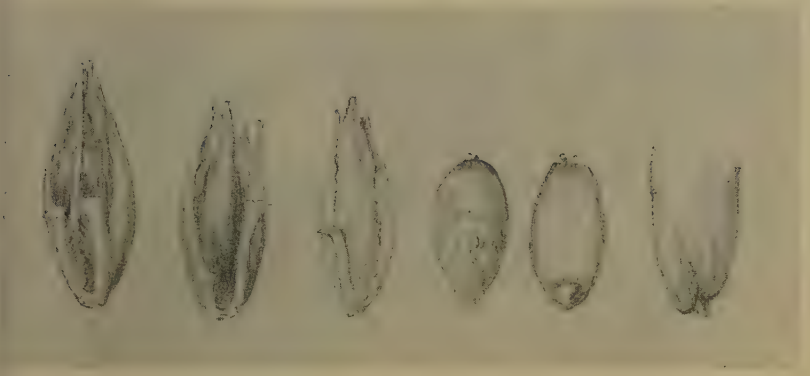

Fig. 46. Johnson grass (Sorghum halepense). Left to right: Spikelets, hulled grains, enlargement of base of grain. x 6. Enlargement x 10.

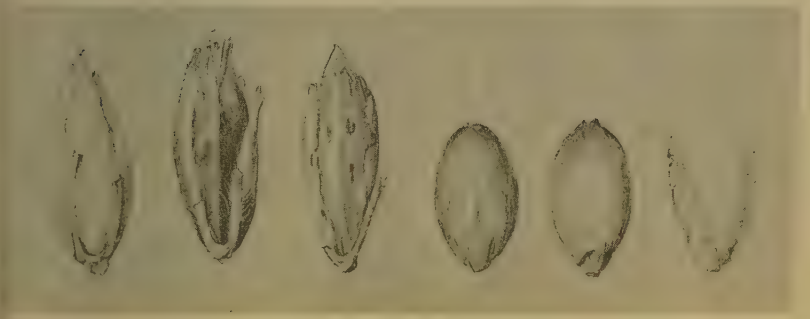

Fig. 47. Sudan grass (Sorghum sudanense). Left to right: Spikelets, hulled grains, enlargement of base of grain. x 5. Enlargement x 8.

REFERENCES

Bellue (1949, 49-50. Comparative descriptions and illustration.
Hillman (1916). Johnson grass and Sudan grass.
_____ and Henry (1945, pl. 1, Figs. 1,2). Illustrations, Johnson and Sudan grass.
U.S.D.A. (1952, 229-230; Figs. 185-186). Seed key and illustrations.
Wright (1951, Gramineae, Fig. 36). Illustration.

Fig. 48. Indian grass (Sorghastrum nutans). Hulled grains. x 10.

LILY FAMILY (Liliaceae)

Two weeds, wild onion (Allinum canadense L.) and wild garlic (A. vineale L.) are noxious. These species reproduce by underground bulbs and aerial scaly bulblets, the latter frequently replacing the flowers in the inflorescence. Seeds are rarely formed; the bulblets are the structures found in agricultural seed.

WILD ONION (Allium canadense L.)
Figs. 49, 50, 51, 52.

Status under law. Prohibited. Mississippi, Tennessee (no botanical name given). Restricted. Alabama, Arkansas, Florida, Georgia, Illinois, Maine, Missouri, Louisiana, North Carolina, Oklahoma, Ohio, Pennsylvania, South Carolina. Secondary noxious. Delaware, Kansas, Massachusetts, New Jersey, Vermont, Virginia.

The above enumeration includes states designating Allium spp. as noxious as well as those specifically indicating Allium canadense to be noxious. (See heading Allium spp. for further discussion.)

How produced. Clusters of bulblets in terminal umbels replace the flowers. Varieties of wild onion which occur on agricultural soil rarely set true seeds.

Size. Mature bulblets various in size, 5-15 mm. long, 4-12 mm. wide. Immature bulblet fragments encountered in agricultural seed may be much smaller.

Description. Complete bulblet ovoid, broadest below middle, abruptly beaked at apex. External scales several in number, dull brown or yellowish, sometimes darkened towards apex, the outermost frequently fibrous. Inner portion of bulb solid, ovoid, often with basal offsets, usually yellowish to dull grey in color. "Embryo" present at basal (broad) end.

Structures found in agricultural seed are extremely varied in appearance, usually consisting only of fragments of the inner portion of the bulblets. Such fragments may be quite small, frequently approximating the size of the seed in which they are found.

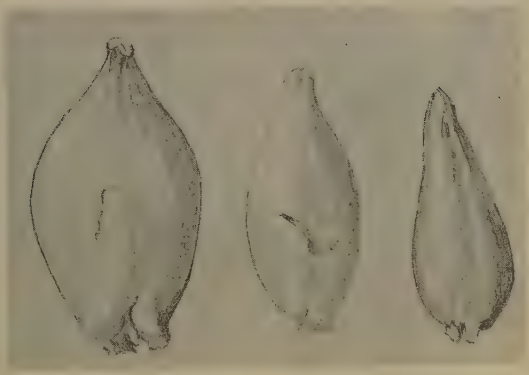

Fig. 49. Wild onion (Allium canadense). Bulblets. x 3.

Fig. 50. Wild garlic (Allium vineale). Bulblets. x 4.

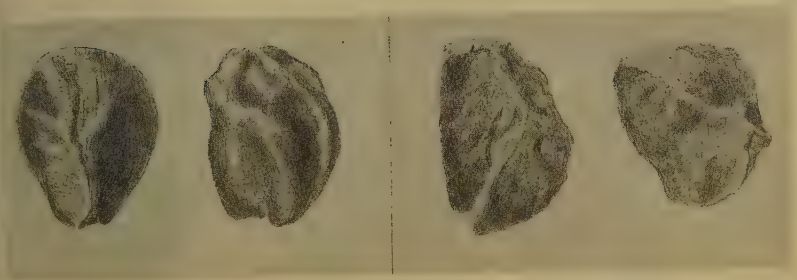

Fig. 51. Allium spp. Seeds. Left: Wild onion (A. canadense); Right: Onion (A. cepa). x 10.

Seeds with which wild onion may be confused.

WILD GARLIC (Allium vineale L.). Bulblet obovoid, broadest above middle. External scale somewhat shiny, frequently yellowish at base and darkened above. (See description of wild garlic, following, for further comparative characters.)

Distribution and importance as a weed. Wild onion is a native perennial, relatively common in eastern North America west to the drier portions of the great plains, and also in the Pacific states. Its weedy characteristics are similar to those of wild garlic (*A. vineale*).

Occurrence in agricultural seed. Wild onion bulblets are most commonly encountered in eastern, central, and southeastern grown forage grass, small grain, and legume (especially crimson clover) seeds.

WILD GARLIC (Allium vineale L.)
Figs. 49, 50, 51, 52.

Status under law. Prohibited. Indiana, Mississippi, Oregon, West Virginia (as wild onion, all crops except orchard grass).[1]

Restricted. Alabama, Arkansas, Florida, Georgia, Maine, Missouri, Ohio, Oklahoma, Illinois, Louisiana, North Carolina, South Carolina,, West Virginia (orchard grass only). Secondary noxious. Delaware, Kansas, Kentucky (as wild onion), Maryland, Massachusetts, Montana (as wild onion), New Jersey, Virginia, Washington (as wild garlic or wild onion), District of Columbia (as wild onion).

The above enumeration includes states designating *Allium* spp. as noxious as well as those specifying *A. vineale*. (See further discussion under heading *Allium* spp. following.)

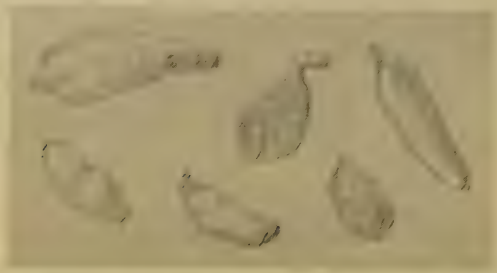

Fig. 52. Wild onion or garlic (probably Allium vineale). Bulblet fragments removed from legume and grass seed. x 4.

How produced. Flowers in terminal umbels but rarely maturing seed, usually replaced by a cluster of bulblets. The bulblets rather than seeds are the propagules usually found in agricultural seed.

Size. Complete bulblets mostly 5-8 mm. long, 2-4 mm. wide. Mature inner bulb 5-7 mm. long but immature ones may be much smaller. Seeds about 2.5 mm. long.

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[1] The West Virginia seed law designates *Allium vineale* as a prohibited weed. However, the regulations allow, in examinations of orchard grass, a tolerance of 10 per 50 grams. Since this exceeds the statistical tolerance of 2, the weed is considered to be restricted.

Description. Complete bulblet broadly to narrowly obovoid, tapering to base, with a twisted beak at apex, consisting of an outer scale and a solid inner portion. External scale somewhat shiny, frequently yellowish at base and darkened above. Solid inner bulb obovoid, spindle shaped or nearly cylindrical, yellow, greyish or dull red dependent upon maturity and subsequent treatment, commonly somewhat withered or contorted. "Embryo" present at extremity of narrowed, basal end.

The true seed (rarely found in agricultural seed) is obovate, laterally compressed with one side convex, the other nearly flat. The attachment scar is laterally placed on one edge of the seed somewhat above the narrowed basal end. Color blue-black, surface somewhat wrinkled and tuberculate.

Seeds with which wild garlic may be confused.

WILD ONION (Allium canadense L.). Bulblet ovoid, broadest below middle. External scales several, dull brown or yellowish, the outermost frequently fibrous. (See description under treatment of wild onion above for further comparative characters.) The bulblets of these plants can ordinarily be distinguished if the entire structure is present; the inner portions can sometimes be identified on the basis of differential shape relative to the position of the embryo, but extremely small bulblets or portions thereof cannot readily be distinguished. Differential identification is rarely necessary, since Allium bulblets, when encountered in seed samples, are universally considered to represent those of noxious species.

Detection and diagnosis. Small bulblets or fragments of bulblets are usually more-or-less shapeless or amorphous in appearance and are sometimes overlooked as inert matter. Contrariwise, material removed from Kentucky bluegrass which superficially appeared to be small bulblet fragments proved, upon further examination, to be excised embryos of wheat (Everson, 1952).

Distribution and importance as a weed. Wild garlic is native to Europe. It is now abundant in the eastern half of North America, and is locally common on the pacific coast. The plant is a perennial reproducing by bulbs, aerial bulblets, and to a limited extent by seed. It is abundant in pastures and meadows as well as in cultivated fields. If eaten by cows, its flavor will taint the milk. The presence of bulblets in wheat subjects the grain to heavy dockage.

Occurrence in agricultural seed. Crimson clover, forage grasses, and small grains from the eastern and southern United States.

REFERENCES

Everson (1952). Discussion and diagrams.
Korsmo (1935, Fig. 263). Illustration and description.
U.S.D.A. (1952, Fig. 220). Illustration.
Wright (1951, Cyperaceae et al. Fig. 6). Illustration.

WILD ONION AND WILD GARLIC (Allium spp.)
Figs. 49-52.

The states enumerated below designate Allium spp. as noxious. The common name designation varies from "Wild Onions" to "Wild Onion and/

or Wild Garlic". We have interpreted these laws, in all cases, as including both Allium canadense and A. vineale.

Prohibited. Mississippi. Restricted. Alabama, Arkansas, Florida, Georgia, Illinois, Louisiana, North Carolina, Oklahoma, South Carolina. Secondary noxious. Delaware, Kansas, Massachusetts, Virginia.

NETTLE FAMILY (Urticaceae)

One noxious species.

WILD HEMP (Cannabis sativa L.)
Fig. 53.

Status under law. Prohibited. Utah.

How produced. The clustered pistillate flowers each mature an achene which is invested within the persistent calyx. Seeds found in agricultural seed are usually represented by the achene, the calyx having been removed in the course of processing.

Size. Achenes 3.0-4.0 mm. long, 1.5-2.5 mm. wide.

Description. Seed elliptic in outline, slightly compressed or biconvex with a narrowly winged margin. Surface smooth, brownish-mottled. Immature seeds greenish-brown, irregularly reticulate with white lines.

Seeds with which hemp may be confused. None.

Distribution and importance as a weed. Hemp is an introduced, Asiatic annual. It is abundantly established on the eastern half of the North American continent, and is sporadic in the west. It does not persist under cultivation, but thrives in rich soil in untended or waste areas. The plant has received considerable publicity as the source of the narcotic, marihuana.

Occurrence in agricultural seed. Hemp seeds occasionally occur in those of oats and soybeans.

Fig. 53. Wild hemp (Cannabis sativa). Achenes, x 15.

REFERENCES

U.S.D.A. (1952, Fig. 225). Illustration.

SMARTWEED FAMILY (Polygonaceae)

The fruit in the smartweed family is an indehiscent achene invested within the persistent flower calyx. The calyx may or may not be destroyed by handling incident to processing, hence, the seed may represent the achene, or achene plus part or all of the calyx hull.

The achene may be three-angled, or flattened and somewhat heart-shaped. The protective pericarp is hard, smooth or finely granular, frequently shiny, brown or black in color. Internally, the seeds contain abundant endosperm and a marginally placed, usually somewhat curved, embryo which extends from the top to the bottom of the seed.

The two principal weedy genera are Rumex, docks and sheep sorrel, among the most common noxious weed contaminants of agricultural seed, and Polygonum, smartweeds. Since analysts frequently find it necessary to identify seeds only as docks or smartweeds, comparative generic diagnoses are presented below.

SMARTWEEDS (Polygonum spp.)
Figs. 54, 55, 61.

Achenes flat or 3-angled with rounded or blunt edges. Surface black or brown--most frequently black in our weedy species (but immature seeds may be brown). Calyx closely investing achene, usually mostly destroyed during processing. Embryo placed in angle of two converging sides.

Two members of this genus are considered noxious, but on a limited basis only. However, since smartweed seeds are abundant in agricultural seed, it is important that confusion with those of dock be avoided.

KNOTWEED (Polygonum aviculare L.)
Figs. 54, 55, 56, 58-67.

Status under law. Restricted. Canada (in lawn and turf grass seed only).

Size. 2.0-2.7 mm. long, 1.0-1.5 mm. wide.

How produced. Axillary flowers mature into achenes closely enclosed by the persistent calyx. These achenes, with or without the calyx, are the seeds of common usage.

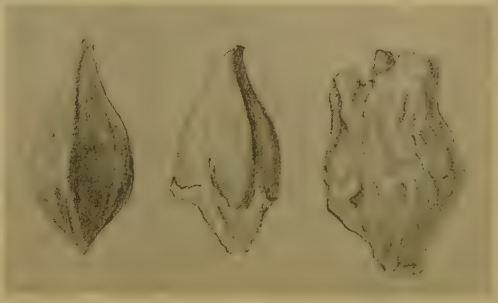

Fig. 54. Knotweed (Polygonum aviculare). Achenes. One on right invested by calyx. x 11.

Description. Achenes three-angled or sometimes only two-edged, ovate-lanceolate in longitudinal view, longer than broad, tapering to an acute point at apex. Angles rounded. Surface dark to light brown depending upon maturity, relatively dull, finely roughened, Torn remnants of calyx adherent to basal portion of achenes, or sometimes major portion of seed covered by closely investing, light-tan calyx.

Cleistogamously formed seeds are occasionally encountered and differ from the above to some extent. They tend to be spindle-shaped, rounded in cross-section, frequently curved or twisted at one end. Their surface is glossy by comparison with seeds typical of this species.

Seeds with which knotweed may be confused.

SMARTWEEDS (Polygonum spp.). Seeds flat or three-angled, the trigonous types generally nearly as broad as long; surface black when mature, brownish when immature, frequently shiny.

The seeds of most species of Polygonum are readily distinguishable from knotweed on the basis of one or several of the above variable characteristics. Seeds of one or two species of the section Avicularia (close relatives of knotweed, which may be doubtfully distinct from that species) appear to have seeds similar to P. aviculare and cannot be distinguished on the above bases. Such seeds will doubtless continue to be identified as knotweed.

Silversheath knotweed seeds, with their broader, dock-like shape and shiny surface are ordinarily easily distinguishable from those of P. aviculare. However, cleistogenes of the latter species (see description above) do possess a relatively shiny appearance and might be confused with silversheath knotweed if careful observation of the shape were not made.

DOCKS (Rumex spp.). Seeds sharp-angled, shiny.

Distribution and importance as a weed. Knotweed, a European introduction, is common throughout the United States and Canada. It is not a weed of cultivated soil but is abundant along paths, roadsides, in tramped or worn lawns. The plant is unusual in its ability to persist in dry soil or gravel, and to tolerate the physical wear of human or vehicular traffic.

Occurrence in agricultural seed. Knotweed seeds occur primarily in seeds of lawn or turf grasses and in various small-seeded legumes.

REFERENCES

Hillman and Henry (1945, pl. 4, Figs. 15-24). Knotweed and similar seeds.

Korsmo (1935, Figs. 1-15). Knotweed and similar seeds, illustrations and descriptions.

U.S.D.A. (1952, Figs. 227-229). Illustrations.

Wright (1951, Cyperaceae et al., Figs. 9-18). Illustrations of knotweed and similar weeds. Color of knotweed (Fig. 10) not natural.

BLACK BINDWEED (Wild buckwheat)(Polygonum convolvulus L.) Figs. 54, 55, 61.

Status under law. Secondary noxious. Alaska

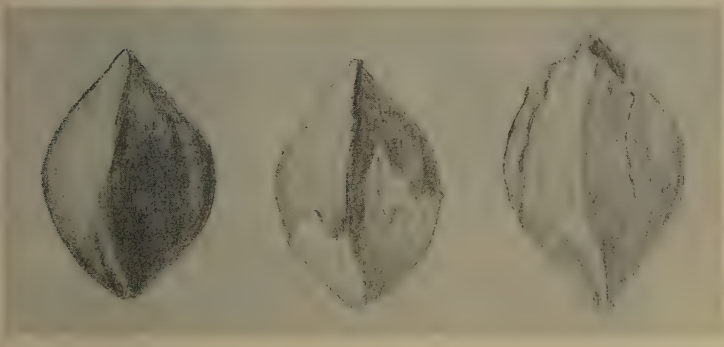

Fig. 55. Wild buckwheat (Polygonum convolvulus). Achenes, with and without calyx hull. x 11.

How produced. Achenes are matured within the persistent calyx. Seeds consist of achenes enclosed within calyx covering, achenes from which part or all of the calyx has been destroyed by processing, and occasionally true seeds hulled out of the enveloping pericarp.

Size. Achenes invested by calyces 3.0-3.5 mm. long, 2.0-2.5 mm. wide; hulled achenes slightly smaller.

Description. Achenes trigonous, usually somewhat rounded at base, pointed at apex, obtuse-angled. Surface black, dull, except for shiny angles, finely granular. Calyx light-brown, close fitting. Hulled seeds (the true seeds) brownish, trigonous.

Seeds with which black bindweed may be confused.

Polygonum spp. Seeds considerably smaller than those of wild buckwheat, except for Hedge buckwheat (Polygonum scandens L.) which has lobes.

Distribution and importance as a weed. Black bindweed, a native of Europe, is common throughout the North American continent with the exception of the southern United States, it is a rapid growing, twining annual which may be common in cultivated soil, grain fields, roadsides, etc.

Occurrence in agricultural seed. Black bindweed seeds are found primarily in those of relatively large-seeded crops, small grains, soybeans, canes (sorghum).

REFERENCES

Hillman and Henry (1945, p. 5, Fig. 2). Illustration.
Korsmo (1935, Fig. 4). Illustration and description.
U.S.D.A. (1952, Fig. 230). Illustration.
Wright (1951, Cyperaceae et al., Fig. 11). Illustration.

DOCKS AND SHEEP SORREL (Rumex spp.)

Achenes trigonous, sharply angled (except for sheep sorrel), shiny brown or reddish-brown. Calyx hulls (except for sheep sorrel) enlarged, brownish-crinkly, loosely fitting about seed, removed by processing except occasionally in seed of small grains. The sheep sorrel hull is tight fitting and frequently not removed by processing. The embryo lies against middle of one of the sides.

Dock seeds may resemble trigonous seed of the following:

SMARTWEEDS (Polygonum spp.). Seeds black or less frequently brown. Edges not sharp angled. Hull when present close fitting. Embryo, in cross-section, located in angle where sides come together.

SEDGES (Cyperaceae). Seeds (achenes) usually not sharply angled. Embryo very small at base of seed, not discernible in cross-section.

The seeds of Rumex are among the most common noxious weed seeds encountered in agricultural seed. Dock is most frequently found in alfalfa, red clover, sweet clover, lespedeza, legume seed mixtures, bluegrass, brome grass, fescues, orchard grass, timothy and oats.

SHEEP SORREL (Rumex acetosella L.)
Figs. 56, 57, 58-60, 62-67.

Status under law. Restricted. Alabama, Arkansas (as Rumex spp.), Florida, Georgia, Indiana (as red sorrel), Louisiana (as Rumex spp.), Mississippi, Missouri, Oklahoma (as red sorrel), South Carolina. Secondary noxious. Iowa, Kansas (as Rumex spp.), Kentucky (as sorrel), Montana, Nebraska (as red sorrel), Washington.

How produced. Flowers dioecious in panicled racemes. Inner sepals persistent and slightly enlarging in fruit. Seed: The achene enclosed within the sepals, the hulled achene, or less frequently (usually in grasses), the achene with pericarp absent.

Size. Hulled achenes 1.1-1.3 mm. long, 0.8-1.1 mm. wide; achene enclosed within calyx, about 1.5 mm. long.

Description. Calyx lobes valvate, close fitting about achenes, coarsely wrinkled or reticulate, persistent or partially destroyed. Achenes shiny brown, trigonous, nearly as wide as long; edges not sharp-angled as in other species of Rumex.

Occurrence in agricultural seed. Seeds of sheep sorrel may occur in those of nearly any small-seeded grass or legume. It appears to be most abundant in bluegrass, fescues, redtop, timothy, red clover and alsike, as well as various pasture and lawn grass mixtures.

Seeds with which sheep sorrel may be confused.

DOCKS (Rumex spp.). Calyx enlarged, loosely fitting about the achene, generally absent on seeds found with those of forage grasses and legumes. Achenes sharp-angled, usually larger than those of sheep sorrel except for size-graded and "rubbed-down" seeds of dock in white and alsike clover.

SCARLET PIMPERNEL (Anagallis arvensis). Seeds irregularly trigonous. Hilum lateral on one of the edges. Outer surface brownish, scurfy-reticulate; when rubbed down, blackish.

Fig. 56. Sheep sorrel (Rumex acetosella). Achenes with and without calyx hull. x 18.

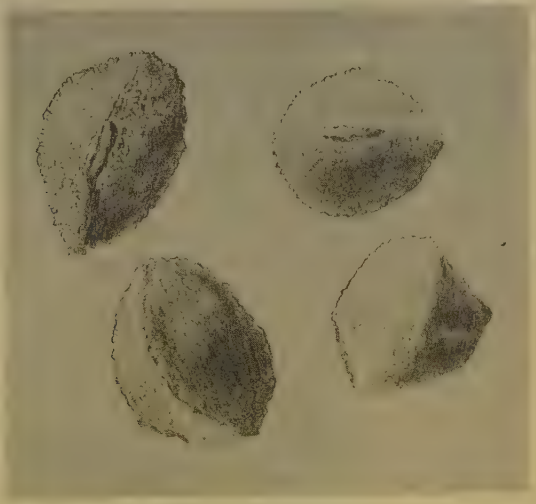

Fig. 57. Scarlet pimpernel (Anagallis arvensis. Seeds. x 22.

Polygonum and Cyperaceae spp. (See generic descriptions of Rumex and Polygonum. pp. 571 and 574).

REFERENCES

Hillman and Henry (1945, pl. 4, Figs. 15-24; pl. 11, Fig. 14). Sheep sorrel and similar seeds.

Isely (1949, 445-446). Comparative descriptions and illustrations.

Korsmo (1935, Fig. 299). Illustration and description.

U.S.D.A. (1952, Figs. 235-244, 541). Illustrations.

Wright (1951, Cyperaceae et al. Figs. 9-18). Sheep sorrel and similar seeds.

SMOOTH DOCK (Rumex altissimus L.)
Figs. 56, 58, 59, 60, 62-67.

Status under law. Restricted. Arkansas, Florida, Louisiana, Mississippi (as Rumex spp.), Missouri, Oklahoma, South Carolina (all as Rumex spp.). Secondary noxious. Connecticut, Iowa, Kansas, Nebraska, New York, Rhode Island, Washington (all as Rumex spp. except Iowa and Nebraska. (See heading Rumex spp., following for interpretation of this generic designation.)

How produced. The flowers are in panicled racemes. The sepals enlarge in fruit and surround the trigonous achene as a loose hull. This hull is usually destroyed in processing. Seeds: the achene enclosed by the calyx, the hulled achene, or (less frequently) the true seed separated from the pericarp.

Size. Achene about 2.5 (3.0) mm. in length, 1.7-2.0 mm. wide: unhulled seed surrounded by calyx lobes 4.0-5.5 mm. in length.

Description. Calyx lobes loosely persistent about achenes, brown, reticulate, entire or nearly so, one valve with a conspicuous dorsal tubercle. Achenes trigonous, shiny-brown, sharp-edged.

Seeds with which smooth dock may be confused.

Polygonum and Cyperaceae spp. (See generic descriptions of Rumex and Polygonum, (pp. 571 and 574).

Rumex spp. (See descriptions of various species for details.) The unhulled achenes can be distinguished on the basis of calyx characters. The hulled achenes are usually slightly smaller than those of smooth dock, but specific identification may be difficult and is frequently unnecessary.

Importance and distribution as a weed. Smooth dock is a native species occurring in the eastern United States west to the Rocky mountains. However, it is most common as a weed in the midwestern states, and is locally abundant in grass fields, small grains, and along roadsides.

Occurrence in agricultural seed. (See generic description, p. 574).

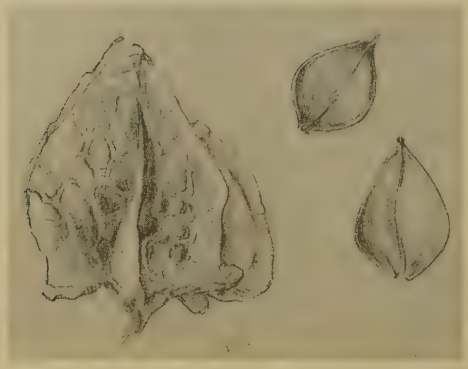

Fig. 58. Smooth dock (Rumex altissimus). Achenes with and without calyx hull. x 9.

REFERENCES

Hillman and Henry (1945, pl. 4, Figs. 15-17). Illustrations, seeds similar to cmooth dock.
Isely (1949, 443-445). Comparative description and illustrations.
U.S.D.A. (1952, Figs. 235-244). Illustrations.
Wright (1951, Cyperaceae et al. Figs. 9-18). Illustrations, seeds similar to smooth dock.

CURLY DOCK (Rumex crispus L.)
Figs. 56, 58, 59, 60, 61-67.

Status under law. Restricted. Alabama, Arkansas (as Rumex spp.), Florida (as Rumex spp.), Georgia, Illinois, Indiana, Louisiana (as Rumex spp.), Mississippi (as Rumex spp.), Missouri, North Carolina, Ohio, Oklahoma (as Rumex spp.), South Carolina (as Rumex spp.), Canada. Secondary noxious. Colorado, Connecticut (as Rumex spp.), Iowa (as sour dock), Kansas (as Rumex spp.), Montana, Nebraska, New York (as Rumex spp.), Rhode Island (as Rumex spp.), Texas, Washington (as Rumex spp.). (See heading Rumex spp., following, for interpretation of generic designation.)

How produced. The one-seeded achene is matured within the enveloping, persistent sepals. Seeds: the unhulled achene (enclosed by the calyx), the hulled achene, or (less frequently), the true seed separated from the pericarp.

Size. Achenes 2.0-2.5 mm. in length; 1.3-1.5 mm. wide; unhulled seed surrounded by calyx lobes 3.0-3.5 mm. long.

Description. Calyx lobes loosely enclosing achene, reticulate; entire or irregularly dentate, one or all three with dorsal tubercles. Achenes trigonous, shiny-brown, sharp-edged. True seeds, separated from pericarp covering, trigonous with a membranous, reddish-brown seed coat.

Seeds with which curly dock may be confused.

SILVERSHEATH KNOTWEED (Polygonum argyrocoleon Steud.). Seeds slightly smaller than those of curly dock, with rounded rather than sharp edges. The position of the embryo within the seed also differs from that of the dock (refer to comparison of Rumex and Polygonum, p. 574). The seeds of silversheath knotweed are likely to occur only in southwestern grown seed, principally alfalfa.

Polygonum and Cyperaceae spp. Compared with docks, p. .

Rumex spp. (See descriptions of individual species for details). The unhulled achenes of Rumex spp. can usually be distinguished on the basis of calyx characters, but it is usually impractical to distinguish hulled achenes.

Distribution and importance as a weed. Curly dock is widely distributed throughout the United States and Canada. It is perhaps most common in the northern states, but is also abundant in California. It does well in a variety of habitats but is of greatest importance in forage legumes and grasses, and small grains.

Occurrence in agricultural seed. (See generic description, p. 574).

Fig. 59. Curly Dock (Rumex crispus). Hulled achenes, and achene enclosed by persistent calyx hull. Broken achene (top right) shows position of embryo against middle of side. Achene, middle right, is without pericarp covering. x 11.

Fig. 60. Curly dock (Rumex crispus). Achenes and cross-section to show position of embryo. x 18.

Fig. 61. Silversheath knotweed (Polygonum argyrocoleon). Achenes and cross-section to show embryo position. x 18.

Fig. 62. Broad-leaved dock (Rumex obtusifolius). Hulled achenes and achene enclosed by persistent calyx. x 11.

REFERENCES

Hillman and Henry (1945, pl. 4, Figs. 15-20). Illustrations, curly dock and similar seeds.
Isely (1949, 443-444). Comparative description and illustrations.
Korsmo (1935, Fig. 233). Illustration and description.
U.S.D.A. (1952, Figs. 227, 235-244). Illustrations.
Wright (1951, Cyperaceae et al., Figs. 9-18). Illustrations, curly dock and similar seeds.

BROAD-LEAVED DOCK (Rumex obtusifolius L.)
Figs. 56, 58-60, 62, 63-67.

Status under law. Restricted. Alabama, Arkansas (as Rumex spp.), Georgia, Louisiana (as Rumex spp.), Mississippi (as Rumex spp.), Missouri (as Rumex spp.), North Carolina, Oklahoma (as Rumex spp.), South Carolina (as Rumex spp.), Canada. Secondary noxious. Connecticut, Kansas, Nebraska, New York, Rhode Island, Washington (all as Rumex spp. except Nebraska). (See heading Rumex spp. following, for interpretation of generic designation).

How produced. See Rumex crispus above.

Size. Same as Rumex crispus above.

Description. Same as Rumex crispus except that calyx lobes are conspicuously dentate with slender teeth. Usually one of the sepals bears an enlarged dorsal tubercle.

Seeds with which broad-leaved dock may be confused.

Rumex spp. (See descriptions of individual species for comparison). It is usually considered impractical to distinguish between the various docks on the basis of hulled seeds, but unhulled seeds can usually be determined by calyx characters.

Polygonum and Cyperaceae spp. See comparison, p 571.

Distribution and importance as a weed. Introduced from Europe, this weed occurs throughout most of the United States and Canada, but is most common in the east. Like the other docks it is perennial from overwintering crowns, and is common in legumes and in forage grasses.

Occurrence in agricultural seed. See page

REFERENCES

Hillman and Henry (1945, pl. 4, Figs. 15-20). Illustrations, broad-leaved dock and similar seeds.
Korsmo (1935, Fig. 234). Illustrations and description.
U.S.D.A. (1952, Figs. 235-244). Illustrations.
Wright (1951, Cyperaceae et al., Figs. 9-18). Illustrations, broad-leaved dock and similar seeds.

WINGED DOCK (Rumex venosus Pursh)
Figs. 56, 58-60, 62, 63, 64-67.

Status under law. Secondary noxious. Nebraska

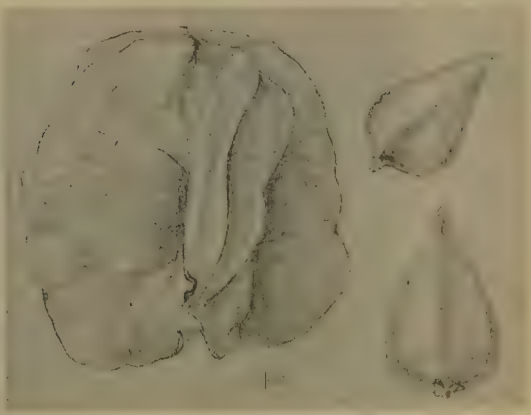

Fig. 63. Winged dock (Rumex venosus). Achene surrounded by persistent calyx and hulled achenes. x 2 and x 3.

How produced. Same as Rumex altissimus above.

Size. Hulls and achenes much larger than those of other species of Rumex. Persistent sepals 1.5-2.0 cm. long, 2.0-3.0 cm. wide. Achene 6.0-8.0 mm. long, 4.0-5.0 mm. wide.

Description. Large persistent sepals rounded, broader than long, reticulate, without tubercles. Achenes trigonous, shiny-brown, sharp-angled, longer in proportion to width than in other species of dock.

Seeds with which winged dock may be confused.

Polygonum and Cyperaceae spp. See page 571.

Rumex spp. Because of the large size of both the calyx hulls and the achenes, seeds of this dock should be relatively easy to identify.

Distribution and importance as a weed. Winged dock, introduced from Europe, differs from the other docks in that it is perennial by creeping rootstock. It is locally abundant in the western Great Plains and Rocky Mountains, and occasional elsewhere.

Occurrence in agricultural seed. See page 574.

REFERENCE

U.S.D.A. (1952, Fig. 245). Illustration.

DOCKS (Rumex spp.)
Figs. 56, 58-60, 62-67.

The states Arkansas, Connecticut, Florida, Kansas, Louisiana, Mississippi, Missouri,[1] Nebraska, New York, Oklahoma, Rhode Island, South Carolina, and Washington list Rumex spp. as noxious without designating kinds. In view of the facts: (1) that correspondence with various of these states has indicated that Rumex spp. is interpreted as meaning

[1]All docks noxious by regulation (Combs, 1952). However, only Rumex crispus and R. acetosella are listed as noxious in the law.

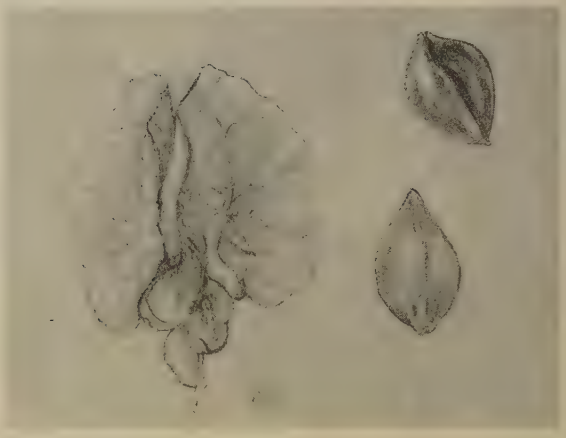

Fig. 64. Rumex acetosa. Hulled achenes and achene surrounded by persistent calyx. x 11.

Fig. 65. Rumex conglomeratus. Hulled achenes and achene surrounded by persistent calyx. x 11.

Fig. 66. Rumex mexicanus. Hulled achenes and achene surrounded by persistent calyx. x 11.

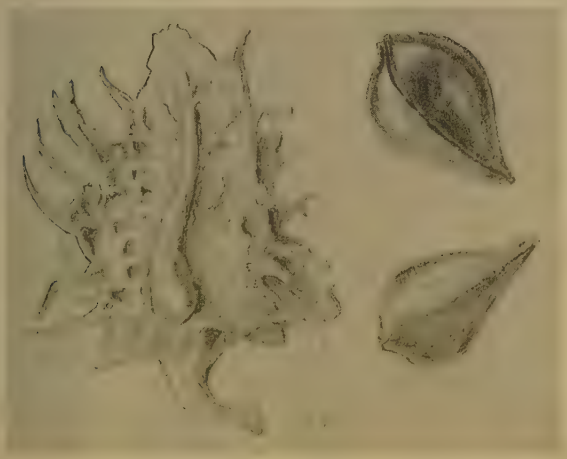

Fig. 67. Rumex pulcher. Hulled achenes and achene surrounded by persistent calyx. x 11.

any species commonly called dock or (literally) any species of Rumex, (2) that hulled achenes of the three most common weedy species (i.e. Rumex crispus, R. obtusifolius, R. altissimus) are rarely distinguished, we have designated these weeds as noxious in all of the above states. It is probably true that Rumex altissimus, occurring primarily in the midwest, is scarcely to be considered a noxious weed in the eastern states, and R. obtusifolius vice versa. However, Rumex achenes in seed shipped in interstate commerce from one of these regions to the other would undoubtedly be considered noxious--regardless of the species or the area of the country in which the plant is usually found.

Other species of Rumex, occasional or frequent as weeds, whose seeds, although not specifically mentioned by any seed law, may be considered to be noxious include the following.

Rumex acetosa L. Hulls without tubercles, broad, not pointed at tip. Achenes about the size of those of curly or broad-leaved dock, frequently blackish-brown with lighter angles.

Rumex conglomeratus Murr. Hulls rather small, tight-fitting, each (sepal) with a very large dorsal tubercle. Achene about 1.5 mm. long, 0.6-0.7 mm. wide. Throughout the United States, occasional or common on wet soil.

Rumex pulcher L. Sepals lacerate-dentate, teeth not longer than width of body. Achenes similar in size to those of curly dock, reddish-brown. Common on west coast, occasional elsewhere.

Rumex mexicanus Meisn. Seeds about the same size as those of curly dock. Sepals entire, each with a narrow tubercle on the back.

REFERENCES

Association of Seed Control Officials of the Southern States. Uniform noxious weed list for southern states. Proc. 8th ann. meeting. p. 102. 1951.

Bellue, M.K. Weed seed handbook, 64 pp. California Dept. Agric. 1949.

Combs, L.L. Personal communication, June 12, 1952.

Everson, L.E. Wild garlic (Allium vineale) bulblets in Kentucky bluegrass. News Letter Assoc. Off. Seed Anal. 26(Feb.):13-15. 1952.

Hartley, Alice. Personal communication, September 12, 1951.

Hillman, F.G. Distinguishing characters of the seeds of Sudan grass and Johnson grass. U.S.D.A. Bull. 406. 1916.

_____, and H.H. Henry. The more important forage-plant seeds and incidental seeds commonly found with them. Reissue. U.S.D.A. Bur. Pl. Industry, Soils, and Agric. Engineer. 1945.

Hitchcock, A.S., and Agnes Chase. Manual of the grasses of the United States. U.S.D.A. Misc. Publ. 200. Rev. 1950.

Isely, Duane. Seeds of Iowa noxious weeds. Iowa State Coll. Bull. P101. 1949.

_____. Seeds of Bromus secalinus and commutatus. Contr. Handb. Seed Test. Assoc. Off. Seed Anal. No. 1. 1951.

_____, Dale West, and R.W. Pohl, Seeds of agricultural and weedy Bromus. Iowa State Coll. J. Sci. 25:531-548. 1951.

_____, and W.H. Wright. Noxious weeds and their seeds: miscellaneous species. Proc. Assoc. Off. Seed Anal. 41:139-144. 1951.

Jones, R.J. Personal correspondence. August 30, 1951.
Justice, O.L., and M.D. Whitehead. Seed production, viability and dormancy in the nut grasses Cyperus rotundus and C. esculentus. J. Agr. Res. 73:303-318. 1946.
Korsmo, Emil. Weed Seeds. 175 pp. 34 pls. Grondahl and Sons, Oslo, Norway. 1935.
Leggatt, C.W. Report of standardized tests committee. Proc. Assoc. Off. Seed Anal. 41:41. 1951.
Musil, A.F. Testing farm seeds in home and school. U.S.D.A. Misc. Publ. 437. 1942.
_____. Seeds of grasses cultivated for forage or occurring incidentally with crop seeds: miscellaneous species. U.S.D.A. Div. Forage Crops and Diseases. 5 pp. 1 pl. 1944.
_____. Seeds of grasses cultivated for forage or occurring incidentally with crop seeds: the genus Panicum. U.S.D.A. Div. Forage Crops and Diseases. 7 pp. 2 pls. 1944a.
_____. Distinguishing characters of the seeds of four species of Agropyron. U.S.D.A. Div. Forage Crops and Diseases. 5 pp. 7 pls. 1946.
_____. Distinguishing species of Avena from their seed. U.S.D.A. Div. Forage Crops and Diseases. 9 pp. 5 pls. 1946a.
_____. Distinguishing seeds of ryegrass, Lolium spp. and the large-seeded fescues, Festuca elatior and varieties. U.S.D.A. Div. Forage Crops and Diseases. 5 pp. 5 pls. 1948.
_____. Seeds of Agropyron intermedium, A. trichophorum, and A. elongatum, compared. U.S.D.A. Div. Forage Crops and Diseases, 1 p. 2 pls. 1948a.
_____. Seeds of Agropyron, eleven species of economic interest compared. U.S.D.A. Div. Forage Crops and Diseases. 6 pp. 6 pls. 1950.
Randall, C.G. The occurrence of red rice in California seed rice. Bull. Calif. Dept. Agr. 39:104-106. 1950.
Seed Trade Buyers Guide. Seed World Publications Inc. Chicago. 1952.
Taylor, P.S. Personal communication, September 5, 1951.
United States Department of Agriculture. Manual for testing agricultural and vegetable seeds. Agr. Handb. No. 30. 440 pp. U.S. Govt. Print. Office, Washington, D.C. 1952.
West, Dale. Seed characteristics of Elymus virginicus L. Proc. Assoc. Off. Seed Anal. 41:109-110. 1951.
Wright, W.H. Weed Seeds. 7 vols. illustrations. Department of Agriculture, Canada. 1950-51.

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IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 587-602

THE HISTOLOGY OF THE BUD GRAFT UNION IN ROSES[1]

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The use of graftage as a means of plant propagation antedates the earliest recorded history. Several methods of graftage, essentially as described in the earliest writings, are still important in horticultural practice. Recent advances made in the production of root-inducing growth regulants have not eliminated graftage as a means of propagation.

Bud grafting, or more popularly, budding, is the accepted method for propagating garden roses at the present time. More than 95 per cent of all roses grown in 1950 were produced in this way. Other methods of propagation, including various forms of whip grafting and cuttage, are used to a limited extent, chiefly in the propagation of roses for greenhouse use.

The histological mechanism of the healing of a graft is known to be based to a large extent upon the activities of the cambium and its derivative tissues. The details have been worked out in the tongue-and-whip graft, but the histology of the bud graft has been virtually ignored.

The growing importance of the phenomenon of stock-scion compatibility has called attention to the possible relationship between the histology of the bud graft union and relative compatibility. The present study was undertaken to determine the course of histological events in the healing of the bud graft of rose, and thereby provide basic information for the further studies in relation to compatibility.

Prior to the beginning of rose breeding in the early nineteenth century, the preferred methods of rose propagation were the rooting of suckering stems and cuttage. Graftage, in its various forms, was used only when the preferred methods failed. This situation was changed by the production of improved rose varieties by European rose breeders. Popular demand for the new rose hybrids necessitated a rapid, efficient method of propagation. By the middle of the century budding had become an accepted method for the propagation of roses in Europe. The practice of propagating roses by budding was slower in being accepted by American nurserymen.

Journal Paper No. J - 2331 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1212.

The author wishes to express his appreciation to Dr. J.E. Sass for assistance in interpretation of the data; to Dr. E. S. Haber for guidance and aid in the undertaking of which this report is a part; to Mr.S.B. Hutton, The Conard - Pyle Co., West Grove, Pennsylvania, for the plant materials used in this investigation.

With the widespread use of budding it became evident that a given variety does not produce the same percentage of "take" on all stocks, and that some varieties do not give a satisfactory "take" irrespective of the stock used.

REVIEW OF PERTINENT LITERATURE

The literature pertaining to graft unions is concerned chiefly with grafting techniques and with the physiological relationships between stock and scion. This phase has been fully reviewed by Roberts (8). The histology of the tongue-and-whip graft has been described by several workers (2, 10).

The first significant work on the bud graft was that of Sorauer (11) who described briefly the activities of tissues in the bud graft. He observed that in the stock the cambium and a layer of cambial derivatives remain attached to the bark flaps which are lifted for the insertion of the scion. If budding is attempted during a period of relative cambial inactivity, separation occurs in such fashion that entire sections of the cambial region, including the youngest phloem cells, remain attached to the wood.

Herse (4) studied the healing of the bud graft in pear and apple. He agreed with Sorauer that the cambium remains attached to the lifted bark flaps. In making his bud grafts, he left a sliver of wood in the scion, which made union possible only around the edge of the scion. Tissues responsible for joining stock and scion were found to be formed by the cambium and by undifferentiated cells in "wood and bark". It is to be inferred that he used the term "bark" to include all tissues external to the cambium, and the term "wood" for all tissues internal to the cambium.

Bailey (1) studied apple bud grafts at weekly intervals through the ninth week after budding. He attributes the formation of callus to the proliferation of the medullary rays of the stock below the bud shield, as well as by the cambium and by xylem parenchyma. Most of the callus was stated to be derived from the stock. In bud grafts made during rapid cambial activity, partially differentiated cells of young xylem proliferate and contribute some of the wound callus.

The relative importance of the cambium and other tissues in the histological development of piece-root grafts of apple has been studied by Sass (9). He found that callus may be derived largely from primary cortex of the scion or from secondary phloem of both stock and scion and, in some cases, most of the callus may be derived from the primary cortex and secondary phloem well outside the cambium. Variable proportions of the callus may be contributed by the tissues of the bark. He concludes that any living tissue located outside the xylem cylinder, with the exception of the periderm, may proliferate to form callus, and the cambium may contribute very little of the callus.

In well-matched grafts Sass described an arc of cambium, continuous with the cambia of stock and scion. This cambial arc results from the activity of bridging cambium which differentiates from the spongy callus adjacent to the old cambium. Differentiation proceeds tangentially until the cambia of stock and scion are connected. The completed cambial cylinder sheathing the union lays down a vascular cylinder which forms the tree's second annual ring.

Edmunds (3), Mansfield (6), and Pearce (7) describe current budding practices in European and American nurseries.

METHODS AND MATERIALS

The rose bud grafts examined during this study were made on the two types of understocks, seedlings, and rooted cuttings, used in commercial production. The principal study was concerned with bud grafts made in true root tissue on seedlings; budded cuttings were used for comparative purposes.

Using the method described by Mansfield (6), six lots of Rosa multiflora seedlings were budded on each of three successive mornings with scions of R. multiflora, Welch strain. Each lot consisted of 25 plants. Beginning the first day of budding one sample was taken at random every day for 14 days from each lot of 25 plants. All bud grafts in which the shield had begun to discolor were discarded. Those in which the bud had shriveled were also discarded.

Rooted cuttings of Odorata 22449 were budded with Odorata 22449 scions, using the same budding and sampling procedures described for R. multiflora seedlings.

The untrimmed samples were killed in a Nawaschin type (Craf II)(10) formula. After three weeks in the killing fluid the bud grafts were trimmed to remove all parts of the understock except a small portion immediately around the bud graft. The grafts were cut in half longitudinally.

The grafts were processed in dioxane-normal butyl alcohol, infiltrated with paraffin, and embedded in Parlax (10). Prior to sectioning, the trimmed blocks were soaked in water at 40° C. for 24 to 48 hours. Samples for sectioning were taken by random selection from the embedded material. Difficulty in securing good adhesion with gelatin adhesive was experienced until a 2 per cent celloidin bath was added to the staining series (5). A safranin-fast green staining combination was found suitable for this material. The plant materials used in this investigation were provided by the Conard-Pyle Co., West Grove, Pennsylvania.

EXPERIMENTAL RESULTS

The present study has confirmed and augmented the observations of experienced horticulturists with respect to the gross features of the bud graft of the rose. In a successful bud graft the shield and the bud retain their normal green color and the bud remains plump. If the petiole is left attached to the shield, as in this study, the behavior of the petiole is indicative of success or failure in healing. In a successful graft the attached petiole gradually becomes yellow, and by the tenth day it drops off cleanly when touched. If the graft fails, the petiole gradually shrivels, becomes black, and adheres tightly to the blackening shield.

In addition to the behavior of the petiole, graft failure is associated with other external gross symptoms. The entire shield may blacken by the tenth day, however, blackening may not become evident until the end of the second week. Discoloration may begin at either the base or the apex of the shield and progresses toward the center. Discoloration of the bud is not necessarily indicative of graft failure if the shield retains its normal green color.

Figs. 1-6. Steps in making a "T" bud graft.

Fig. 1. Stock in condition for budding.

Fig. 2. Stock prepared for insertion of scion.

Fig. 3. Bud stick showing two types of scions. "a", is used for dormant bud sticks; "b", for bud sticks from shoots.

Fig. 4. Scion prepared for insertion into stock. "a", apex of shield; "b", bud or "eye"; "c", base of shield.

Fig. 5. Scion in position in stock.

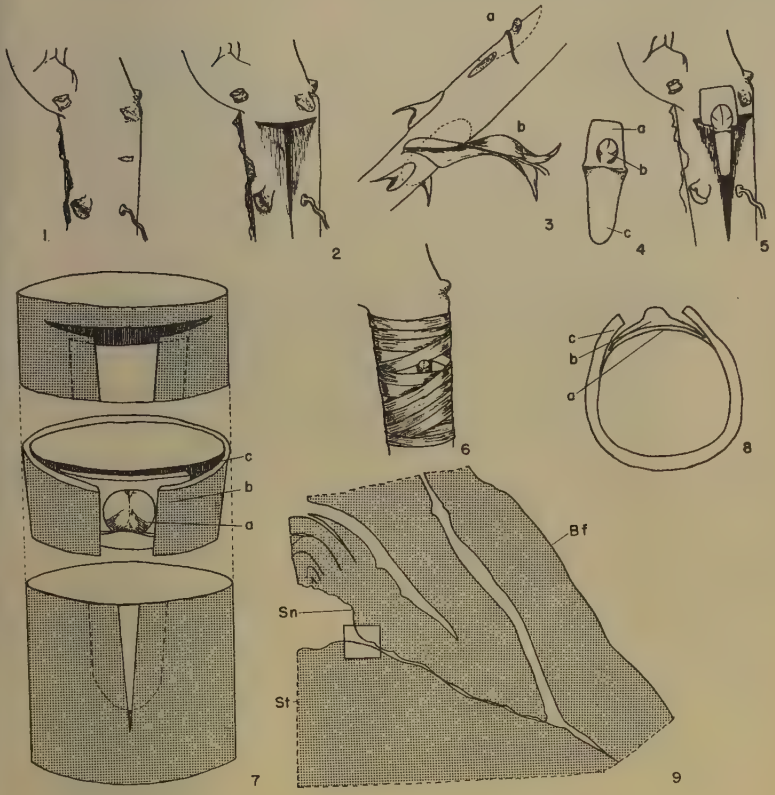
Fig. 6. Completed bud graft tied with rubber budding strip.

Fig. 7. Diagram of bud graft, showing parts discussed in this paper. "a", scion, "b", wounded surface of stock; "c", bark flap.

Fig. 8. Cross-section through the bud of the scion of the bud graft diagramed in Fig. 7. "a", scion; "b", wounded surface of stock; "c", bark flap.

Fig. 9. Orientation drawing for Fig. 10. 34x. "Bf", bark flap; "Sn", scion; "St", stock.

Fig. 10. Detail of designated zone of Fig. 9. Reactivation of cells on the injured surfaces of the stock and scion on the third day after budding, 600x. "Sn", scion; "Ne", necrotic plate; "Pr", phloem ray; "Xr", xylem ray; "In", stock-scion interface; "St", stock.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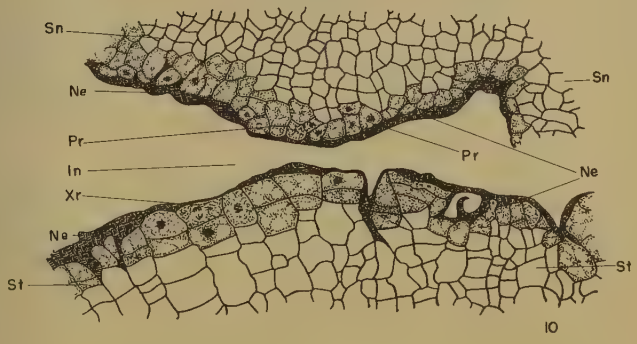
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Fig. 11. Orientation drawing for Fig. 12. 34x. "Bf", bark flap; "Sn", scion; "St", stock.

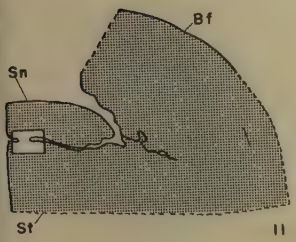
Fig. 12. Strand of coalesced calli on the fifth day after budding. 600x. "Sn", scion; "Ne", necrotic plate; "St", stock; "Pr", phloem ray; "Xr", xylem ray; "Cc", coalesced calli.

Fig. 13. Orientation drawing for Fig. 14. 34x. "Bf", bark flap; "Sn", Scion; "St", stock.

Fig. 14. Stratified callus derived from immature secondary xylem in material collected eight days after grafting. 600x. "Bc", callus derived from scion tissue; "Ne", necrotic plate; "Xp", proliferated xylem.

Fig. 15. Orientation drawing for Fig. 16. 34x. "Bf", bark flap; "Sn", scion; "St", stock.

Fig. 16. Bridging cambium linking intact stock cambium with developing cambiform tissue in material collected three days after budding. 600x. "Ne", necrotic plate; "Ca", intact stock cambium; "Cb", bridging cambium; "Cf", cambiform tissue; "Xp", proliferated immature secondary xylem; "Bf", bark flap.



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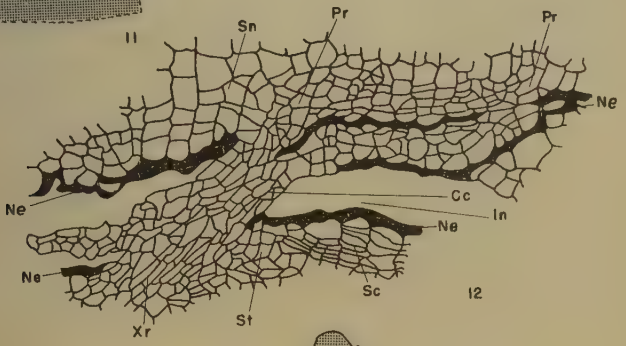

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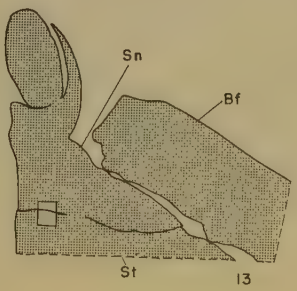

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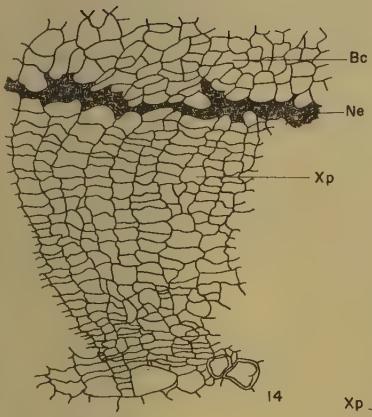

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The tenacity with which the shield adheres to the stock is indicative of the potential success of the union. Considerable pressure is required to separate some buds from the stock as early as five days after the graft is made, whereas other buds are readily removed at this age. Observations on subsequent dates indicate that the buds which expand and grow are firmly adherent; whereas buds which fail to grow are still loosely attached.

Perceptible swelling of some of the buds is evident by the fifth day, and such buds may produce a shoot by the end of the second week. Swelling of the bud is usually a reliable criterion of successful union.

Some grafts develop profuse callus over the outer surface of the scion, usually the lower half. This condition does not seem to be correlated with ultimate success or failure of the union.

The gross features of the bud graft are shown in Figs. 7 and 8. These diagrams show the relationship of the scion to the components of the stock in a typical bud graft at the completion of the budding process. The middle portion of the bud (Fig. 7), which contains the scion bud, is of greatest interest in this study. Fig. 8 is a cross section through this part of the bud graft.

The process of making the bud graft injures tissues of both stock and scion. In cutting the scion from the bud stick, the active cambium and newly formed secondary cells are destroyed. Similar injury occurs in the cambial zone of the stock. The brittle, succulent nature of the tissues of both the stock and scion at the time of grafting is conducive to extensive tearing of tissues in the cambial zone. When the flaps of bark on either side of the "T" incision are raised, tearing and separation of stock tissues occurs in such manner that the cambium and young cambial derivatives are destroyed. No trace of intact cambium was found in either the bark flaps or on the cut surface of the stock at the time of grafting.

Within two days following budding, the injured cells form a plate of dry, dead, necrotic tissue which covers the cut surfaces of the scion and stock. By the third day, cell division begins in the uninjured cells bordering on the surface of the wound behind the necrotic plates. Between the second and fourth day, the terminal cells of the broken xylem rays and immediately adjacent cambial derivatives on the cut surface of the stock enlarge and divide as in Fig. 10. Repeated tangential division produces callus strands of large, thin-walled cells, one to five cells wide. These strands rupture the plate of necrotic tissue covering the face of the stock, and the callus protrudes into the stock-scion interface (Fig. 12).

Formation of callus strands also occurs from terminal cells of broken phloem rays and from the immediately adjacent immature secondary phloem on the cut surface of the scion. After rupturing the plate of necrotic tissue and protruding into the stock-scion interface, the callus strands continue to proliferate parallel to the face of the scion and extend further into the space between stock and scion. Radial and tangential cell divisions increase the callus strands derived from the xylem rays and phloem rays of the stock and scion, respectively.

The terms xylem ray and phloem ray are used here to designate the recently formed, highly meristematic, cambial derivatives in radial continuity with the older, fully differentiated xylem and phloem rays, respectively.

A different type of callus develops behind the necrotic tissue which covers the cut surfaces of the stock and scion. The cambial derivatives, or immature secondary xylem cells, on the cut surfaces of the stock between the rays, also proliferate and form callus which does not rupture the necrotic plate behind which it develops. Repeated tangential division produces callus characterized by concentric arcs of rectangular, thin-walled cells. Stratified callus of this type is shown in Fig. 14. As this callus increases in size, fragments of necrotic tissue are pushed further into the stock-scion interface until contact is made with an opposing necrotic fragment or with callus tissue. Merging of stock and scion calli is obstructed in localized areas by these fragments of necrotic tissue.

As the callus strands derived from the xylem rays of the stock and the phloem rays of the scion increase by tangential and radial division, they tend to lose their strandlike character. The stock and scion calli eventually coalesce, and further identification of callus with respect to its source is no longer possible. Contact between stock and scion calli is complete by the fifth day (Fig. 12).

Near the end of the second week the stock-scion interface fills with callus, derived principally from the proliferating immature secondary xylem and immature secondary phloem of the stock and scion, respectively. During this period, discrete groups of cells, especially those derived from immature xylem, enlarge, and their walls thicken and begin to lignify. Isolated cells in the merged stock-scion calli become greatly enlarged and resemble immature vessels. However, no marked secondary thickening or lignification of the walls was noted at this age. The callus strand depicted in Fig. 20 contains several of these vessel-like cells.

During the second week after grafting, short arcs of cambiform tissue, two to four cells wide and three to five cells long, appear in the calli that had originated from the immature secondary phloem of the scion and the immature secondary xylem of the stock. These cambiform arcs elongate by radial division of the callus cells at the edges. Cambiform cells that were derived from the scion callus, extend across the zone of merged callus and connect with cambiform cells that were undoubtedly derived from stock callus (Figs. 18 and 19).

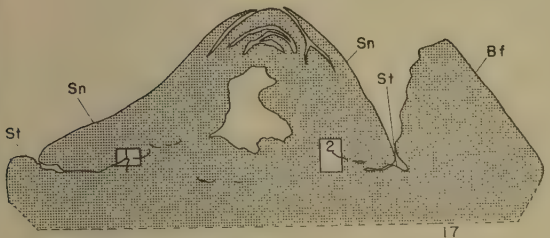
Formation of callus tissue under the bark flaps of the stock on either side of the scion follows the same developmental pattern observed in callus formation between the stock and the scion. By the third day, a plate of necrotic tissue had formed over the torn surfaces of the stock and bark flaps. Terminal cells of broken xylem and phloem rays adjacent to uninjured stock tissue enlarge and divide tangentially and form strands of callus, which rupture the necrotic plate and protrude into the fissure between the torn tissues. After emerging from the ruptured necrotic plate, the strands turn in the direction of the scion and develop parallel to the torn surfaces of the bark flaps and stock. Cell reactivation in the broken phloem and xylem rays begins first in those rays adjacent to uninjured stock tissues and progresses toward the scion.

Callus is produced by tangential proliferation of the immature secondary phloem and secondary xylem on the torn surfaces of the bark flap and stock, respectively, behind the necrotic plate. This callus is composed of arcs of meristematic, stratified cells which, in combination

Fig. 17. Orientation drawing for Figs. 18 and 19. Area 1 refers to Fig. 18; area 2 refers to Fig. 19. 25x. "Bf", bark flap; "Sn", scion; "St", stock.

Fig. 18. Differentiating arcs of cambiform tissue in proliferated immature secondary phloem ten days after budding. 600x. "Ne", necrotic plate; "Sc", stock callus; "Cc", mature callus strand; "Cf", cambiform tissue.

Fig. 19. Completed cambiform arc linking stock and scion. 600x. "Ne", necrotic plate; "Cc", mature callus strand; "Cf", arc of cambiform tissue; "Sc", stock callus; "Bc", scion callus.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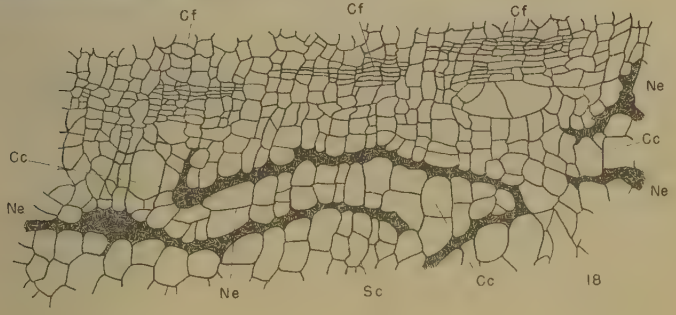
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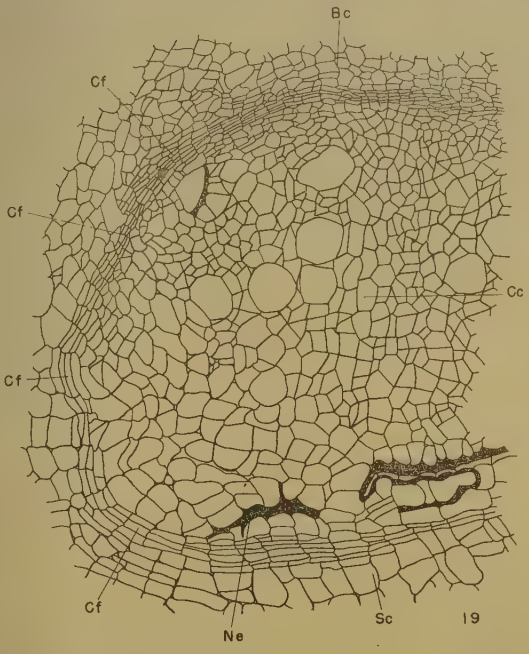
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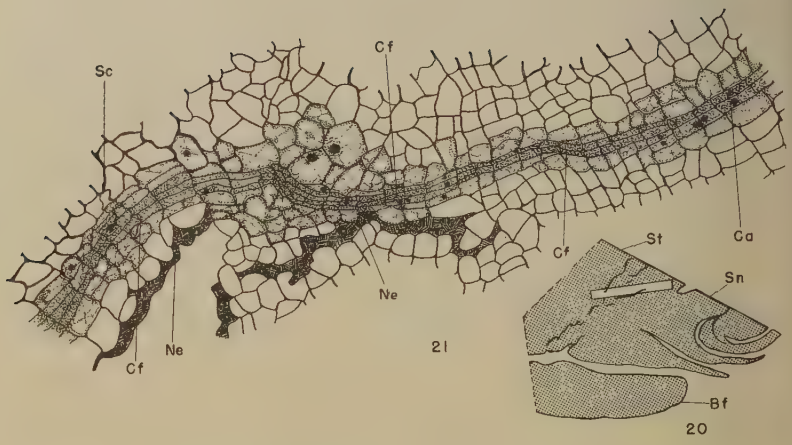

Fig. 20. Orientation drawing for Fig. 21. 34x. "Bf", bark flap; "Sn", "St", stock.

Fig. 21. Portion of reconstituted cambial cylinder. 600x. "Ne", necrotic plate; "Ca", cambium of the bud; "Cf", cambium derived from cambiform tissue; "Sc", stock callus.

with ray callus, fills the fissure between the stock and bark flaps. The cells arising out of early proliferation of the secondary xylem are small, thin-walled, have a dense protoplasm, and are highly stratified. Cells arising out of later proliferation are larger, have less dense protoplasm, and have less pronounced stratification.

By the tenth day an uninturrupted band of cambiform tissue, joined to the uninjured cambium of the stock on either side of the scion, extends across the face of the stock. This tissue differentiates from cells of the stratified callus formed by the early proliferation of the secondary xylem. Tangential division of cells in this part of the xylem-derived callus near uninjured stock tissue produces an arc of cambiform tissue, two or three cells wide. This arc elongates in the direction of the scion by radial division of callus cells at its edge and effects junction with arcs of cambiform tissue originating in stock callus under the scion. Between the third and fifth day after grafting, spongy callus cells adjacent to uninjured stock cambium undergo repeated tangential and radial division and form a short arc, three to four cells wide. This arc of tissue bridges the callus between intact stock cambium and the band of cambiform tissue developing across the face of the stock (Fig. 16).

During the differentiation of bridging cambium and of the band of cambiform tissue in the stock on either side of the scion, the cambium of the bud of the scion begins to extend by radial division of the cells at the edges. The developing cambial cylinder of the bud, usually following the inner surface of the scion, effects union with cambiform tissue extending out of stock callus through the zone of merged callus. This union of cambial elements of the stock and scion occurs between the tenth and fourteenth day and forms a continuous ring of cambium through the scion and around the stock (Fig. 21).

The necrotic plate of tissue which forms over the cut surface of the stock and scion is relatively thin, but it becomes very thick at the edges of the bark flap. This enlarged necrotic portion is formed not only of cells injured in making the stock incision, but also of uninjured cells adjacent to them. By the fifth day a layer of cork cambium had formed from tangential division of cells immediately behind the necrotic ones. The cork cambium extended across the bark flap, cutting off the dying tip. Between the tenth and fourteenth day an arc of cork cambium had developed, cutting off the edge of the scion. Periderm does not extend into callus tissue.

DISCUSSION

From the present studies and from discussion with large-scale rose propagators, it has become evident that success or failure of a bud graft can be determined within two weeks after budding. A bud may grow within ten days after budding if the top of the stock is progressively cut back. Growth of the bud between the second and third weeks also occurs as a result of the ringing effect of the constricting bud ties. By interference with the translocation of food, and possibly of growth-inhibiting substances, the net effect of constricting ties is similar to that produced by heading back the stock.

Early stock-scion callus contact has a bearing upon the practice of

rooting budded cuttings. It explains the higher bud "take" of cuttings made five days after budding, than in cuttings taken earlier. The merging of stock and scion calli between the fourth and sixth day affords morphological verification for these empirical observations.

The general pattern of histological events in the development of the rose bud grafts studied in this investigation agrees with that reported by earlier workers (11, 4, 1, 10). However, differences were observed which can be ascribed to the technique of scion removal from the bud stick, difference in plant materials used, or to difference in age of the bud grafts studied.

The tearing of tissues in the zone of young cambial derivatives below the cambium of the stock is in agreement with the observations of Sorauer (11), Herse (4), and Bailey (1); however, contrary to their reports, no intact cambium was observed on the lifted bark flaps. The tearing occasioned by the lifting of the bark flaps destroys the cambial cylinder in the vicinity of the stock incision. This agrees with Sorauer's (11) observation that the tenderest tissues at the time of grafting are the ones torn in preparing the stock for the insertion of the scion.

The results of this investigation are in accord with Sass' (10) description of callus formation in tongue-and-whip grafts of the apple in that the cambium is of minor importance as a contributor of callus. In the bud graft, the cambium at the extremities of the torn area of the stock does not form callus. The callus is contributed by cambial derivatives in scion, stock, and bark flaps, with the greatest portion of the callus being derived from stock tissues. The traditional statement that the cambium is the chief contributor of callus is not borne out by these studies.

It is unfortunate that earlier workers (1, 11) did not describe more fully the technique used for removing the scion from the bud stick. Differences in the scion removal technique used in this and previous investigations could provide a partial explanation for the disparities discussed earlier in this paper. Herse's (4) method of removing the scion from the bud stick, which involves the removal of a sliver of wood with the bud, has more in common with a form of grafting called side-grafting rather than with bud grafting.

The formation of a plate of necrotic tissue from cells injured in the grafting process over the wounded area of the stock, scion, and bark flaps and its subsequent rupturing and malformation by developing callus, is in agreement with Herse's (4) and Bailey's (1) observations.

Bailey's (1) report, that extensive callus formation occurs over the entire interface of the shield and that extensive proliferation of stock callus occurs from rays below the scion, is not verified by this study. Callus formation from the scion occurs only in the area under the bud.

The arcs of cambiform tissue which develop during the tenth to fourteenth day in the proliferated immature secondary xylem and phloem of the stock and scion, respectively, are similar to the bridging cambium described by Sass (10). The bridging cambium he described is formed from spongy callus adjacent to old cambium and eventually connects the cambia of the stock and scion. These cambiform arcs appear to be a transition stage in the development of permanent cambium. The union of scion cambium with that of the stock isolates arcs of cambiform tissue, which may be the source of the unlignified whorls of callus tissue reported by Bailey (1).

Contrary to the descriptions of some earlier investigators, the healing of torn tissues in the stock on either side of the scion, especially the injured tissues of the bark flaps, is not intimately concerned with the success or failure of the graft. The present work has shown that cambial continuity between stock and scion is effected through the proliferated immature secondary xylem of the stock and secondary phloem of the scion, rather than through the merged calli of bark flap and wood of the stock. The chief contribution made by the flaps of bark on either side of the scion, as well as the upper and lower portions of the bud shield, is mechanical. These parts serve to hold the scion firmly in place until union has taken place.

The healing of the bud graft union in rose occurs in a shorter time than in apple. Bailey (1) states that in apple bud grafts union of cambial elements of stock and scion takes place between the third and fourth week after budding. Union of stock and scion cambia of the rose is complete by the fourteenth day. In view of the wide difference between apple and rose in the time required for union, the apple bud graft deserves re-examination.

SUMMARY

A study was made of the histology of the healing process in the bud graft of the rose.

The process of making the "T" incision in the stock and lifting the flaps of bark, and the process of removing the scion from the bud stick, destroys the cambium of the areas involved. Cambium does not contribute callus during the healing process.

The callus involved in the healing of the bud graft is derived from immature, recently derived, secondary phloem and secondary xylem in the immediate vicinity of the scion bud.

Cambial continuity between stock and scion is established by bridging cambium derived from proliferated callus of the stock and scion, respectively. This cambium establishes continuity with the intact cambial cylinder of the bud.

Cambial union between stock and scion is completed by the fourteenth day. The reconstructed cambial cylinder is capable of functioning in a normal manner.

A successful bud graft union is dependent upon simultaneous differentiation of cambiform tissues in adjacent calli of stock and scion, and upon the union of the cambium of the bud with the bridging cambium developed in the calli.

REFERENCES

1. Bailey, J.S. A microscopic study of apple graft union. Unpublished M.S. thesis. Ames, Iowa. Iowa State College Library. 1923.
2. Bradford, F.C., and G.B. Sitton. Defective graft unions in the apple and pear. Michigan State Agr. Exp. Sta. Tech. Bull. 99. 1929.
3. Edmunds, Fred. Rose budding in America. Gard. Illust. 66:211. 1949.
4. Herse, F. Beiträge zur Kenntnis der Histologischen Erscheinungen bei der Veredlung der Ostbäume. Landw. Jahrb. 37:71-136. 1908.

5. Johansen, Donald E. Plant Microtechnique. 1st ed. New York, N.Y. McGraw-Hill Book Co., Inc. 1940.
6. Mansfield, T.C. Roses in Colour and Cultivation. New York, N.Y. E.P. Dutton and Co. 1940.
7. Pearce, S.A. The art of budding. Gard. Illust. 66:138-139. 1949.
8. Roberts, R.H. Theoretical aspects of graftage. The Bot. Rev. 15: 423-463. 1949.
9. Sass, John E. Botanical Microtechnique. 2nd ed. Ames, Iowa. The Iowa State College Press. 1951.
10. _____. Formation of callus knots on apple grafts as related to the histology of the graft union. Bot. Gaz. 99:364-380. 1933.
11. Sorauer, Paul K.M. Manual of Plant Diseases. Vol. 1. Non-parasitic Diseases. Tr. by Frances Dorrance. 3rd ed. Wilkes-Barre, Pennsylvania. The Record Press. 1914.

IOWA STATE COLLEGE JOURNAL OF SCIENCE
Vol. 28 No. 4 May 15, 1954 pp. 603-621

INITIATION AND DEVELOPMENT OF THE INFLORESCENCES OF PHALARIS ARUNDINACEA L. AND DACTYLIS GLOMERATA L.[1]

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The gross aspects of the flowering cycles of important agronomic perennial grasses are readily observable, and the time and duration of anthesis, as well as the approximate date of seed maturity are well known to plant breeders and seed producers. The time of initiation of the inflorescence, and the details of floral development are of interest in connection with experimental treatment of breeding material. Field observations of the flowering cycle do not provide precise information on these morphological processes. The present study was conducted to determine the time of inflorescence initiation, the sequence of organ initiation, and the histological derivation of floral organs in two perennial grasses, Phalaris arundinacea L. and Dactylis glomerata L.

REVIEW OF PERTINENT LITERATURE

The gross developmental morphology of the shoot apex of the grasses has received considerable attention during the past three decades. Recent investigations have stressed the cytohistology of the grass shoot apex during the initiation of the inflorescence and during the subsequent development of the floral organs.

Payer (21) studied the development of the floral apex with special emphasis on the development of the floral organs, primarily the carpel and anthers in Panicum aduncum, Triticum monococcum, Ehrarta panicea, and Stipa juncea. His descriptions are classics in the developmental morphology of the species that he studied.

Wolff (33) showed that new leaves and the tissues of the stem are derived from the delicate tip of the shoot. He applied the term "punctum vegetationis" to the growing point. Foster (15) has suggested that the term "growing point" is inaccurate and has replaced it by the term "shoot apex".

The shoot apex of angiosperms is highly variable in form and dimensions. Sharman (28, 29, 30, 31, 32) has described the shoot apex of several grasses and placed apices in three categories: (a) the long type which bears from 12-20 leaf primordia, (b) the intermediate type with from 5-10 leaf primordia, and (c) the short type with only one or two leaf primordia.

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[1] Journal Paper No. J - 2301 of the Botany and Plant Pathology Section and Farm Crops Subsection, Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1001.

[2] The author is indebted to Dr. John E. Sass for assistance in conducting this study and wishes to express his appreciation to Dr. C.P. Wilsie for granting access to the grass breeding nurseries at the Agronomy Farm of the Iowa State College.

Abbe, Phinney and Baer (1, 2) have recently studied the shoot apex of Zea with respect to the quantitative characters of the external and internal features of growth, from the seedling stage to the time of initiation of the inflorescence. They determined the number of days required for the initiation and organization of a phytomer, the structural unit of the grass shoot. Their observations on growth patterns was used to explain the "maximal" and "minimal" areas described by Schmidt (27), who first studied the process of rhythmical alternation of the shoot apex during successive plastochrochrones.

The study of the zonal structure of the shoot apex began with the early cytohistological investigations of Hanstein (17). He proposed the classical "histogen theory" which described three initial concentric zones in apices of the root and shoot. These zones were designated the "plerome", "dermatogen" and "periblem". According to Hanstein the three histogens are derived from separate sets of initial cells. The epidermis is subsequently derived from the dermatogen, the cortex from the periblem, and the vascular system and pith from the plerome. This terminology is adequate for the histogens in the roots of both monocots and dicots, but it is not accurate for the zonation in the shoot because the distinction between periblem and plerome is not always evident in the stem.

Schmidt's (27) tunica-corpus theory, in contrast to that of Hanstein, recognizes two major zones, (a) the tunica, which is designated as the external layer or layers of the shoot apex, in which anticlinal planes of cell division maintain the mantle-like layers and (b) the corpus, in which the planes of cell division are commonly at random.

The number of tunica layers in shoot apices varies widely in grasses. Sharman (31) has described the shoot apex of Agropyron repens (L.) Beauv. as having three mantle-like layers, which he has designated as the dermatogen (the outer layer), the hypodermis, and the subhypodermis, all of which enclose a central core.

Studies of floral histogenesis began with the work of Grégoire (16). He maintained that there is no tunica-corpus zonation in floral apices and that floral apices differ in this respect from the vegetative apex. He described the floral apex as consisting of a "massive parenchymateux", enveloped by an extensive mantle, "manchon meristematique". The planes of cell division were described as periclinal in the mantle, which gives rise to floral organs, to vascular traces and to the central corpus.

Foster (13, 14, 15) has given a critical review of the previous work on shoot apices, with special reference to the zonal structure of the floral apex and fluctuations in the number of tunica layers.

The grasses are particularly favorable subjects for the study of the shoot apex. Rösler (25) studied leaf and bud initiation in Triticum vulgare Host., and stated that the leaf arises solely from "dermatogen", although he was not certain whether the cells beneath this layer also contribute to the sheath and lamina.

Kliem (19) observed that in Avena sativa some of the inner leaf mass is derived from the corpus. Douliot (9) studied the annular swellings on the shoot apices of Phragmites communis Trin., and found that both the "epidermis and the underlying cells" play a part in the derivation of this annular meristem which he interpreted as the future sheath.

Bugnon (8) showed that in the grasses a new primordium originates on

one side of the apex by periclinal divisions in the "epidermis" and underlying cells. Similar activity around the apex from the point of initiation forms an annular meristem which he interpreted to be the lamina.

Sharman (31) concluded that leaf primordia can first be detected by periclinal divisions in the "dermatogen" cells on one side of the shoot apex. He was not certain whether these divisions are preceded by similar divisions in the second layer of the tunica, the "hypodermis". He states that the internal tissue of the young leaf is derived from both the dermatogen and the hypodermis, whereas the subhypodermis and core contribute nothing. He found that only anticlinal divisions occur in the outer layers of the shoot apex at the point of initiation of a bud primordium. The "core" of the bud is derived from the "subhypodermis" of the main axis.

Evans and Grover (12) have reviewed the literature on the early development of the inflorescence of grasses. They studied floral development in eight species (10, 11), in seven tribes. They found that the transition from the vegetative to the flowering phase can be recognized by the appearance of swellings or protuberances on the apex, followed by the accumulation of rudimentary phytomers, which eventually produce a cylindrical shoot apex.

Bonnet (4, 5, 6, 7) described the transformation of the vegetative apex into an inflorescence, and the subsequent development of successive orders of branches and floral organs in several grasses. The order of differentiation of flower parts is: in Triticum, glumes, lemma, anthers, palea and pistil; in Hordeum, glumes, lemma, palea, anthers, and pistil; in Avena, glumes, lemma, anthers, palea, lodicules, and pistil; and in Zea, glumes, lemma, palea, anthers, and pistil.

Knobloch (20) noted that in Bromus inermis Leyss. the spikelet contains from 2-10 florets, and the ovary is tricarpellate and uniloculate.

Philipson (24) stated that in Agrostis canina L. "proliferated spikelets are found in autumn", and that in "proliferated spikelets only the lemmas develop into leaves". He found that the order of development of the floret organs is lemma, stamens, palea, lodicules, and pistil.

Sass and Skogman (26) have studied the initiation of the inflorescence in Bromus inermis Leyss. They found that transition from the vegetative to the flowering phase occurs from early- to mid-April at Ames, Iowa, and that complete transformation of the stem apex into a floral axis occurs within two weeks after the transition is initiated.

MATERIALS AND METHODS

Two perennial grasses were used in this study. Collections of Dactylis glomerata L., orchard grass, and Phalaris arundinacea L., reed canary grass, were obtained from clonal plots at the Agronomy Farm of the Iowa State College, Ames, Iowa.

Samples of 25 growing points from three different clones of D. glomerata and P. arundinacea were collected on November 16, 1950. Both fresh and preserved materials were used in the diagnosis of the condition of the stem apex. Additional observations were made on these two grasses on February 3, 1951 and March 15, 1952.

Regular sampling was begun on April 6, 1951. A sample of 12 apices

was collected for each grass on this date, and subsequently at four day intervals. Alternate clones of P. arundinaceae and D. glomerata, growing under natural conditions along a drainage way of a railroad, were sampled to supplement the material collected from clones in the breeding plots at the Agronomy Farm. Portions of the sods were washed free of soil and the rhizomes were traced back to the crown to insure the proper identification of each shoot (Hitchcock 18), (Bennet 3), This precaution was necessary because the sods were contaminated with other perennial and annual grasses.

The growing points of each sample were excised by splitting the fresh sprouts down the center and removing the portion above the node of the largest expanded leaf. The excised apices were evacuated in an Allen-Bouin killing solution (Formula II), and stored pending further processing. A second killing and fixing solution, the Erliki-Zirkle formula for basic fixation-image, was used for cytological studies during the critical periods of floral initiation. A third preservative, a modified FAA-glycerin formula, was used to maintain a stock of material for dissection under a stereoscopic binocular microscope.

Tissues preserved in the Allen-bouin and Erliki-Zirkle formulas were processed and embedded in paraffin. All material was sectioned serially at eight microns and stained in safranin-fast green for diagnostic and histological studies. Sections used for making projection drawings or photomicrographs were stained in hemalum-safranin, or stained in hemalum for a few minutes prior to staining in safranin-fast green.

Median longitudinal sections and transverse serial sections were used for the diagnosis of stage of development from the vegetative phase through the transition of the flowering phase.

Line drawings and diagrams were prepared from projected outlines of excised material and from serial sections. Excised specimens were suspended in a glycerin medium on a hanging-drop slide. The details of each diagram were then filled in on each outline drawing by observations under the stereoscopic binocular microscope at a magnification of 90 diameters.

EXPERIMENTAL RESULTS

Phalaris arundinacea L.

The dormant vegetative apex. Phalaris arundinacea L. is a perennial grass that produces culms from rhizomes. The vegetative apices from which the culms arise are enclosed in firm scaly buds, located in the axils of papery bracts along the main axis, and at the apex of the rhizome. The vegetative apices in the buds of late summer and fall are the potential flowering apices of the culms of the next growing season. The apices in the dormant condition are relatively short and may contain from seven to ten foliage leaves and three or more scale leaves. The number of foliage leaves in the dormant bud is not constant.

Histogens. The vegetative apex of the bud is a short, smooth dome. The tunica consists of two layers which maintain their identity and continuity by anticlinal cell division. The corpus consists of a homogeneous zone of polygonal cells in which cell division occurs in random planes. From four to six leaf primordia are present in late autumn and persist

through winter dormancy (Fig. 1). The dimensions of such apices range from 70μ to 98μ in width in a transverse plane above the last-produced leaf primordium, from 182μ to 308μ in a transverse plane at the base of the oldest leaf primordium, and from 196μ to 238μ, measured vertically from the axil of the oldest leaf to the apex.

Provascular strands arise early in the ontogeny of a leaf. Vascular elements in the outermost three scale-leaves are well developed in the dormant buds. These scale leaves remain underground and do not differentiate a sheath or lamina. The next two leaves, produced in acropetal order above the scale leaves, are foliage leaves. These leaves, when dormant, have two to three protoxylem elements at a level through the plane of the last leaf primordium. Little or no internodal expansion occurs in the bud until spring.

Initiation of the inflorescence. The initiation of the inflorescence in Phalaris arundinacea L. occurred during the period April 6, to May 2, 1951. The initiation phase is recognized by a marked elongation of the apical meristem into a long tapered axis (Figs. 2 and 3). At this stage, foliage primordia continue to be laid down in acropetal, distichous order, and five to eight leaf primordia are produced rapidly. Leaf initiation can be detected histologically by cell enlargement and by anticlinal divisions of one or more cells in both layers of the tunica. This activity is followed by a periclinal division in the second layer of the tunica, and subsequently followed by two adjacent periclinal divisions in the first layer of the tunica (Fig. 1). A slight protuberance forms as a result of this activity. Lateral propagation of activity around the stem raises a crescent shaped ridge, which finally becomes a complete ring, or annular meristem. Continued activity in the second layer of the tunica and in the peripheral layer of the corpus gives rise to the central mass of the leaf, first by many periclinal cell divisions, followed by cell division in random planes. Meristematic activity along the top of the annular meristem produces an extended free edge which becomes the leaf margin. Growth of the margin is more rapid at the point of leaf initiation and the young leaf soon arches over and remains very closely appressed to the apex.

Early in the transition from the vegetative to the flowering phase, the initiation of leaf primordia becomes retarded, and a marked change in histogen activity takes place in the axils of leaf primordia high on the axis of the apex. At this time a morphological transition takes place and the resulting apex is no longer identifiable as a vegetative apex (Fig. 2). Rapid anticlinal cell division in the tunica layers is preceded by periclinal divisions in the peripheral layer of the corpus, in the axil of a bract primordium (Fig. 5). These zones of activity produce the primordia of branches of the first order. No periclinal divisions occur in the two layers of the tunica at the position of the first-order branch primordia; therefore these two layers are derived directly from the tunica (Figs. 6-8). The bract subtending each branch primordium shows a two-lipped profile when viewed in longitudinal section (Fig. 3). The bracts are initiated in the same manner as leaves, but become less prominent in the apical region as the period of inflorescence initiation draws to a close. By the time the seventh to the ninth first-order branch primordium is produced, the subtending bract may be a rudiment of three or four projecting cells, or the bract may be absent.

The apex continues to lay down first-order branch primordia, until the time of spikelet initiation. Simultaneously, second- and third-order branch primordia are laid down in rapid order, beginning at the base of the inflorescence and moving at a rapid pace in each first-order branch, toward the distal end of the inflorescence. The number of branch primordia produced on each first-order branch decreases acropetally and the inflorescence becomes pyramidal (Fig. 13).

The organ initiation of each succeeding branch-order is the same as that outlined for the first-order branch primordia. The elongation of the branches is retarded until after spikelet primordia have been initiated. The length of such an inflorescence at this stage of development may be from 2.8 to 4.0 mm., measured from the last-produced foliage leaf. Accelerated growth and expansion of the branches proceeds with the development of floral primordia.

Development of floral organs. Each branch-order ultimately terminates in a spikelet. The first spikelet to be initiated was observed on the apex of the inflorescence in material collected May 2, 1951. First and second glume primordia are initiated in rapid order and are followed by the initiation of the sterile bract primordia in acropetal order. The first indication of glume initiation is in the second layer of the tunica (Fig. 9). The glumes are derived solely from the two tunica layers. The two sterile bracts are initiated in the tunica and derive a portion of their central mass from the peripheral layer of the corpus (Fig. 10). The lemma of the fertile floret is initiated in the same manner as a leaf primordium. The terminal floret is initiated simultaneously with the fertile lemma primordium (Fig. 11). The lemma primordium of the terminal floret may or may not be initiated (Figs. 4 and 12). Abortion in the terminal floret can be detected very early in the meristematic dome by the rapid vacuolation of cells of the tunica and corpus. Thus, the distal floret remains as a vestigial, lignified mass of cells compressed between the palea and the subtending sterile bract. The initiation of the fertile lemma is followed by the appearance of stamen primordia, which arise as 3 small papillae on the dome of the floret primordium. One stamen is initiated in the same plane as the lemma, whereas the other two stamens arise at lateral positions on the dome. Stamen initiation is followed by the initiation of the palea (Fig. 12). The palea is produced by the tunica and is derived without noticeable activity in the corpus. The carpel primordium is initiated last, in the same plane as the fertile lemma. The carpel is produced on the dome in the same manner as a foliage leaf. Growth is more rapid at the point of initiation and the carpel margins soon envelope the young ovule, which is produced from the residual dome of apical meristem (Fig. 4). Two lodicule primordia are initiated in the axil of the lemma below the axillary stamen. This marks the end of floral organogeny in the spikelet.

The order of development of floral organs in Phalaris arundinacea L. is lemma, stamens, palea, pistil and lodicules.

Dactylis glomerata L.

The dormant vegetative apex. Dactylis glomerata L. is a perennial grass which produces aerial shoots from buds in axils of the older leaves. This plant has no rhizomes and is maintained from season to season by

virtue of the winter hardiness of the "crown", an extensive cluster of vegetative sprouts which are produced after the flowering season.

The apex of each overwintered shoot may produce an inflorescence during the next growing season. If the apex is killed by freezing, the oldest axillary bud becomes the potential flowering culm. Collections of apices for each sample were restricted to the apices of the main axis. The apices in the dormant condition are somewhat short and may contain from three to five leaf primordia and from four to five well developed foliage leaves. The oldest leaves of a sprout are killed by freezing. The number of leaves present on the axis is not constant and the degree of development of the last-produced leaf primordium is not constant.

Histogens. The vegetative apex of the shoot is relatively long and terminates in a smooth dome (Fig. 14). The apex is laterally flattened when viewed in transverse section. The tunica consists of two mantle-like layers, which maintain their identity and continuity by anticlinal cell division.

An occasional periclinal division has been observed in the second layer at the distal end of the apex. The corpus consists of a homogeneous zone of polygonal cells in which cell division occurs in random planes. The dimensions of apices during the dormant period range from 98μ to 112μ in width in a transverse plane above the last-produced leaf primordium, from 210μ to 280μ in a transverse plane at the base of the oldest leaf primordium, and from 238μ to 280μ measured vertically from the axil of the arched-over leaf to the apex.

Provascular strands arise early in the ontogeny of the leaf. As many as three xylem elements are evident in the third leaf when viewed in transverse sections at the plane of the last-produced leaf. Successively older leaves contain protophloem and protoxylem cells in increasing numbers and the number of strands in each leaf also increases. Internodal expansion and leaf initiation begin when favorable temperatures occur in early spring.

Initiation of the inflorescence. The initiation of the inflorescence in Dactylis glomerata L. occurred during the period April 12 to May 2, 1951. The initiation phase is first recognized by accelerated growth in the apex, which elongates rapidly and produces from four to six foliage leaf primordia in acropetal, distichous order (Fig. 15). The dimension of apices range from 112μ to 140μ, measured across the apex above the last produced leaf primordium, from 284μ to 364μ across the base of the apex at a level with the axil of the leaf that is arched over the apex, and from 350μ to 434μ in a vertical plane measured from the axil of the arched-over leaf to the apex. Leaf initiation can be detected histologically by cell enlargement and anticlinal divisions of a few cells in both layers of the tunica (Fig. 14). Periclinal cell divisions occur later in the second layer of the tunica, followed by periclinal divisions in the outer layer. The peripheral layer of the corpus and the second layer of the tunica contribute to the central mass of the leaf. Periclinal cell divisions in both layers proceed for a short time and give rise to radial rows of cells. Lateral propagation of activity around the stem raises a crescent shaped ridge, which finally becomes an annular meristem. The meristematic margin of the leaf becomes very active early in leaf ontogeny, and as a result of this activity the leaf elongates rapidly in the region of the mid-rib and soon arches over the shoot apex.

The transition from the vegetative to the flowering phase is initiated by increased activity in the formation of branch primordia, and transition is simultaneously accompanied by decreased activity in the initiation of new leaf primordia. Rapid anticlinal division in the tunica is preceded by periclinal division in the peripheral layers of the corpus, all occurring in the axil of a bract primordium (Fig. 16). The two layers of the tunica in the new branch are derived directly from those of the apex of the main axis, and this tunica maintains its continuity by anticlinal cell division. A two-lipped profile is evident in the first three to five leaf primordia, viewed in longitudinal section. Bract primordia become less evident in the distal end of the inflorescence as initiation of first-order branches is terminated.

From 9 to 15 first-order branch primordia may be produced in the inflorescence before second-order branches appear on the most proximal branches. At this stage of development the inflorescence becomes bifacial (Figs. 24-26). All second-order branches project to one side of the flattened inflorescence (Fig. 26). Succeeding orders of branches are produced similarly, and all floral organs are initiated on one side of the main axis. The number of branch primordia produced in each first-order branch decreases acropetally and the inflorescence becomes a flattened cone.

The organogeny of each succeeding branch order is the same as that outlined for the first-order branch primordia. The elongation of branches and expansion of the inflorescence is retarded until after the initiation of spikelet primordia. The length of such an inflorescence at this age ranges from 2.2 to 3.0 mm., measured from the axil of the last-produced foliage leaf. As spikelet initiation proceeds in basipetal order, the growth and elongation of the branches subtending each spikelet is retarded, and the final result is a cluster of fascicled spikelets.

Development of floral organs. Every branch of the panicle ultimately terminates in a spikelet. Spikelet initiation was observed in material collected on May 4, 1951. Glume primordia are initiated first in the most distal portion of the apex. The sequence of spikelet initiation is the reverse of the sequence of branch-order and leaf initiation. The first and second glume primordia are initiated in rapid succession (Fig. 21), followed by the initiation of the lemma of the first floret. Simultaneously, the primordium of the second floret becomes initiated. The tunica consists of two layers during organogeny of the spikelet. Glume initiation is restricted to these two layers and the corpus contributes nothing to the central mass of a glume. The initiation of lemma primordia follows the pattern described previously for the foliage leaves.

Successive florets are initiated in acropetal order, and each is subtended by a lemma primordium (Figs. 17, 22, 23). The spikelet of Dactylis glomerata L. is few-flowered. The number of floret primordia ranges from 4 to 6, but only three to four florets produce an ovary. The spikelet is laterally compressed and is typical of the tribe Festuceae. Abortion of the first floret primordium occurs occasionally (Fig. 17). The histogens lose their normal appearance and activity and become necrotic with residual substances piling up in the intercellular spaces.

The first organs to be initiated in the floret are the three stamens. They arise as small papillae on the sides of the dome of the floret primor-

dium. One of the papillae arises opposite the rachilla and the other two at lateral points on the sides of the meristematic dome, all at the same level. Simultaneously, activity in the tunica adjacent to the rachilla gives rise to the palea primordium, which is the product of only the two-layered tunica (Fig. 17). The first detectable sign of the lodicule primordia, on the side opposite the palea, is the appearance of a protuberance on either side of, and below the anther primordia, in the axil of the lemma.

The pistil primordium arises first as a short dome in the center of the floret apex in the plane of lemma initiation. By lateral proliferation, the primordium becomes crescent shaped, and eventually annular. This meristematic ring grows more rapidly from the point of initiation, and thereby becomes oblique. The ovule is derived from the residual meristematic dome which becomes elevated on one side by activity in the region opposite carpel initiation. Continued growth of the marginal meristem soon closes the suture of the ovule. The order of development of the organs of the floret is lemma, stamens, palea, lodicules, and pistil.

DISCUSSION

The precise details of the seasonal cycle of the vegetative and flowering phases has been established in only a few grasses. In the annuals, the time of initiation of the inflorescence obviously occurs during the current season.

There is limited information on floral initiation in the perennial grasses. The present study has shown that the shoot apex is vegetative during dormancy in Phalaris arundinacea L. and in Dactylis glomerata L., and Sass and Skogman (26) have shown the same condition in Bromus inermis Leyss. There is no overwintering of inflorescences in any of the perennial grasses investigated. It is probable that in the geographic area of the present study, perennial grasses follow the above vegetative and flowering cycle.

The vegetative growing points differ in size, form and leaf number in the two grasses studied. P. arundinacea has a short rounded dome (Fig. 1), whereas, D. glomerata has a relatively long flattened dome, and these two species have 4 to 6 leaf primordia on the shoot apex. Structurally these grasses fit into Sharman's classification of apices into long and intermediate types.

The two grasses exhibit the same sequence of initiation of foliar and floral organs, in acropetal, distichous order. The corpus contributes to the mass of a branch and also to the leaf and some to the floral organs. Sharman (30) stated that leaf initiation takes place in the outer two layers of the "dermatogen" and "hypodermis" (= tunica?), and that no leaf tissue is derived from the "subhypodermis and core" (= corpus?). The tunica-corpus terminology is much more precise than the terms used by Sharman, and is in keeping with current usage by most workers in this field.

The change in histogen activity during the transition from the vegetative to the flowering phase in grasses has not been previously described in detail. The present study has shown that the critical histological events of transition can not be observed in gross dissections of apices, and that microtome sections must be studied to obtain a reliable diagnosis of transition.

Basipetal initiation of spikelets occurs in the two grasses studied, and the sequence of organogeny in the floret is lemma, stamens, palea, pistil and lodicules. Bonnett (4, 5, 6, 7) has shown that variation exists in the sequence of organ initiation within the group of annual grasses which he described. Two of these grasses, Triticum and Avena show the same sequence of organogeny as the perennials described in the present study. However, in Hordeum and Zea, the palea arises in advance of stamens. In Avena and Dactylis, the pistil and lodicules arise simultaneously. The sequence does not seem to be consistent within tribes of grasses, and it will be necessary to study many genera to determine phylogenetic trends in floral ontogeny.

Knobloch (20) considered the pistil of Bromus inermis Leyss. to have a tricarpellate and uniloculate structure. The ontogeny of the carpel in the grasses of the present study does not show a tricarpellate condition. On the contrary, the carpel develops as a single foliar structure and it is uniloculate at maturity.

The glumes in both grasses do not show a foliar relationship in their histological derivation.

SUMMARY

A study was made of the initiation and development of the inflorescence of two perennial grasses, Phalaris arundinacea L., and Dactylis glomerata L. The approximate date of floral initiation has been established and the histology of organogeny during the vegetative and flowering phases is described.

The vegetative phase persists through dormancy in the shoot apex in both grasses. The transition period extends from early- to mid-April.

The transition is indicated by retardation of leaf initiation and an acceleration of panicle branch initiation. This detail of transition is not evident in dissections.

Spikelet initiation begins at the distal end of the inflorescence and proceeds basipetally. The sequence of floral organogeny is acropetal in the two grasses, except that in D. glomerata the lodicules and pistil arise simultaneously. The terminal floret aborts in both grasses.

The tunica of the stem apex is a two-layered mantle over the homogeneous corpus.

Foliage leaves, lemmas, stamens and pistils are derived from both tunica and corpus. Initiation of these organs can be detected by periclinal divisions in the corpus.

Glumes, palea and lodicules are derived exclusively from the tunica.

The single carpel is foliar in its ontogeny. The ovule is derived from the residual dome of the floret primordium and becomes elevated and bent inward on the adaxial side of the carpel.

REFERENCES

1. Abbe, E.C. and B.O. Phinney. The growth of the shoot apex in maize; External features. Amer. J. Bot. 38:737-743. 1951.
2. _____, _____ and D.F. Baier. The growth of the shoot apex in maize: Internal features. Amer. J. Bot. 38:744-751. 1951.

3. Bennett, Hugh. The identification of 76 species of Mississippi grasses by vegetative morphology. Miss. Agr. Exp. Sta. Tech. Bull. 31. 1950.
4. Bonnett, O.T. Development of the barley spike. J. Agr. Res. 51: 451-457. 1935.
5. _____. Development of the wheat spike. J. Agr. Res. 53:445-451. 1936.
6. _____. Development of the oat panicle. J. Agr. Res. 54:927-931. 1937.
7. _____. Development of the staminate and pistillate inflorescence of sweet corn. J. Agr. Res. 60:25-38. 1940.
8. Bugnon, P. L'Appareil conducteur chex les graminées. Mem. Soc. Linn. deNormandie 26:21-40. 1924.
9. Douliot, H. Recherches sur la croissance terminale de la tige et de la feuille chex les graminées. Ann. Sci. Nat. Bot. Ser. VII 13: 93-102. 1891.
10. Evans, M.W. The flowering habits of timothy. Amer. Soc. Agron. J. 8:299-309. 1916.
11. _____. The life history of timothy. U.S.D.A. Bull. 1450. 56 pp. 1927.
12. _____, and F.O. Grover. Developmental morphology of growing point of the shoot and the inflorescence in grasses. J. Agr. Res. 61: 481-521. 1940.
13. Foster, A.S. Problems of structure, growth and evolution in the shoot apex of seed plants. Bot. Rev. 5:454-470. 1939.
14. _____. Comparative studies on the structure of the shoot apex in seed plants. Bull. Torr. Bot. Club. 68:339-350. 1941.
15. _____. Practical Plant Anatomy. 2nd ed. N.Y., D. van nostrand Co. 1949.
16. Grégoire, V. La morphogénèse et l'autonomie morphologique de l'appareil floral. I. Le carpelle. La Cellule. 47:287-452. 1938.
17. Hanstein, J. Die Scheidezellgruppe im Vegetationspunkt der Phanerogamen. Festschr. Niederrhein. Gesell. Natur. Heilkunde. 109-143. 1868.
18. Hitchcock, A.S. Manual of the grasses of the United States. 2nd ed. Revised by Agnes Chase. U.S.D.A. Misc. Pub. No. 200. 1950.
19. Kliem, F. Vegetationspunkt und Blattanlage bei Avena sativa. Beitr. Biol. Pfl. 24:281-310. 1937.
20. Knobloch, Irving W. Development and structure of Bromus inermis Leyss. Iowa State College J. Sci. 19:67-98. 1944.
21. Payer, J.B. Traité d'organogénie comparée de la fleur. Paris, Masson. 1857.
22. Peterson, M.L. and W.E. Loomis. Effects of photoperiod and temperature on growth and flowering of Kentucky bluegrass. Plant Physiol. 24:31-43. 1949.
23. Philipson, W.R. The morphology of the lemma in grasses. New Phytol. 33:359. 1934.
24. _____. The development of spikelet in Agrostis canina L. New Phytol. 34:421. 1935.
25. Rösler, P. Histologische Studien am Vegetationspunkt von Triticum vulgare. Planta 5:28-60. 1928.

26. Sass, J.E., and Jane Skogman. The initiation of the inflorescence in Bromus inermis (Leyss.). Iowa State College J. Sci. 25:513-519. 1951.
27. Schmidt, A. Histologische Studien an phanerogamen Vegetationspunkten. Bot. Arch. 8:345-404. 1924.
28. Sharman, B.C. A periclinal division in the dermatogen of the tip of the maize growing point. Nature 146:778. 1940.
29. _____. Developmental anatomy of the shoot of Zea mays L. Ann. Bot. N.S. 6:245-282. 1942.
30. _____. Shoot apex in grasses and cereals. Nature 149:82. 1942.
31. _____. Leaf and bud initiation in the Gramineae. Bot. Gaz. 106-269-289. 1945.
32. _____. The biology and developmental morphology of the shoot apex in the Gramineae. New Phytol. 46:20-34. 1947.
33. Wolff. C.F. Theoria Generationis. (1759) tr. by Dr. Paul Samasaa, Leipzig, Wilhelm Engelmann. 1896.

PLATE I

Fig. 1. Longitudinal section of the vegetative shoot apex of Phalaris arundinacea L., with four leaf primordia. (260x).

Fig. 2. Longitudinal section of a pre-transitional shoot apex of P. arundinacea, prior to the emergence of the branch primordia. (75x).

Fig. 3. Longitudinal section of a transitional shoot apex of P. arundinacea. Note the two-lipped profile of branch (br) and subtending bract (bt). (73x).

Fig. 4. Longitudinal section of a young spikelet of P. arundinacea. All floral organs have been initiated. The terminal floret primordium consists of vacuolated cells which indicate early abortion. First glume (gl_1); second glume (gl_2); first sterile lemma (lm_1); second sterile lemma (lm_2); first fertile lemma (lm_3); terminal floret (fl); ovule (ov); palea (pa); carpel (ca); stamen (st). (164x).

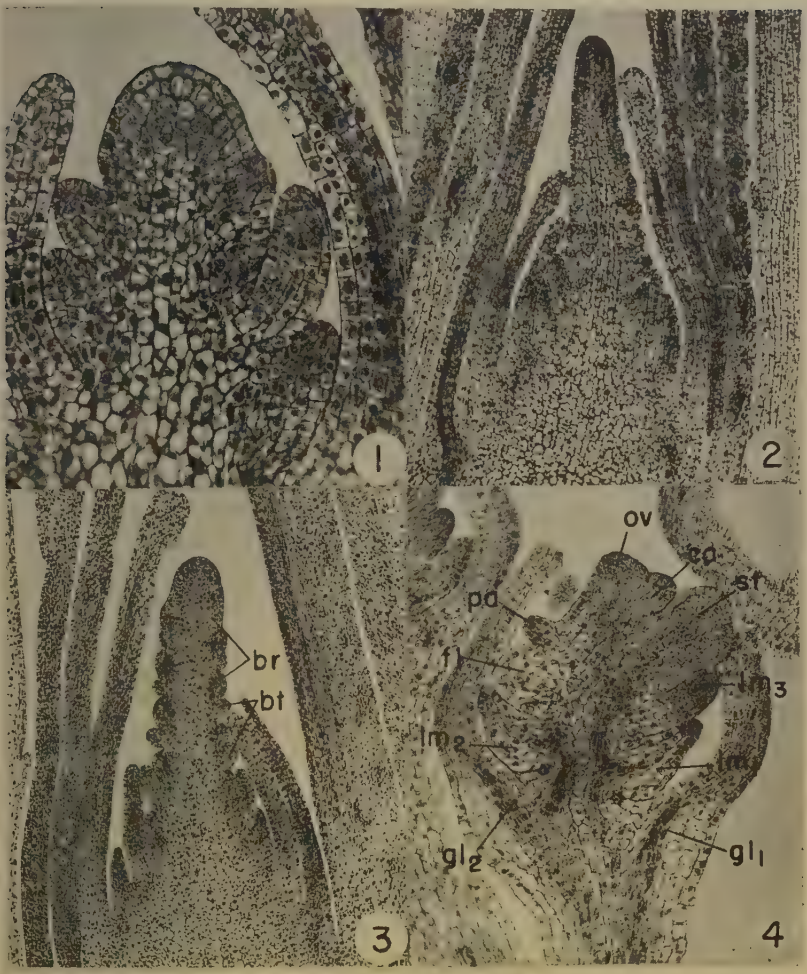
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PLATE II

Figs. 5-8. Diagrams of the transition to the flowering phase in Phalaris arundinacea L. The heavier lines delimit the 2 tunica layers. Branch (br); Bract (bt).

Fig. 5. Longitudinal section of a transitional apex. Branch and bract primordia are recognizable by the mode of cell division. (215x).

Fig. 6. Diagram of a transitional shoot apex in longitudinal section to show area (a) and (b), enlarged in Figs. 20 and 21. (36x).

Fig. 7. Area (a) from Fig. 6, which shows a lag in leaf initiation activity and the radial rows of cells produced by increased activity in the corpus of the branch primordium (br); bract (bt). (215x).

Fig. 8. Area (b) from Fig. 6, which shows histogen activity in the branch and bract below that shown in area (a). (215x).

Figs. 9-12. Diagrams of longitudinal sections of the spikelet in Phalaris arundinacea L. in successive stages of organogeny. The heavy lines delimit the tunica layers. First glume (gl_1); second glume (gl_2); lemma (lm); first floret (fl_1); second floret (fl_2); palea (pa); carpel (ca); stamen (st). (215x).

Fig. 9. Glume initiation in a spikelet with the early stages of the initiation of the first sterile lemma.

Fig. 10. Initiation of the second sterile lemma, fertile lemma, subterminal floret and the terminal floret primordia.

Fig. 11. Advanced stage of spikelet initiation. Note the decline in activity in the histogenesis of the terminal floret.

Fig. 12. Stage of spikelet initiation older than that shown in Fig. 11. The anther primordium is increased in size and the first signs of carpel histogenesis are evident in the plane of the fertile lemma.

Fig. 13. Inflorescence of Phalaris arundinacea L. at the time of initiation of second-order branches. (100x).

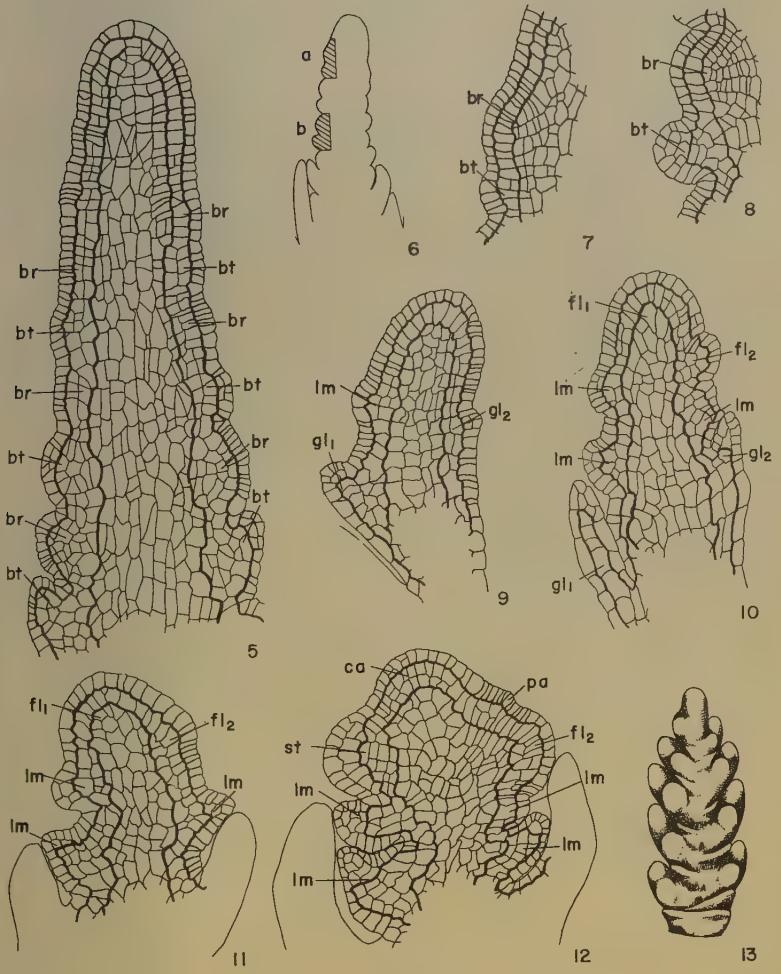
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PLATE III

Fig. 14. Longitudinal section of the vegetative shoot apex of Dactylis glomerata L. from material collected in early April. Note the elongate apical dome and the zones of activity at successive levels of leaf initiation. (260x).

Fig. 15. A pre-transition shoot apex of D. glomerata, with five leaf primordia in longitudinal section. (73x).

Fig. 16. Longitudinal section of a transitional shoot apex of D. glomerata, which shows the emergence of first-order branches. Bract (bt); branch (br). (50x).

Fig. 17. Longitudinal section of a young spikelet of D. glomerata, the first floret has aborted. Note the necrotic appearance of the floret dome and the residual substances in the intercellular spaces. First glume (gl_1); second glume (gl_2); florets (fl_3 and fl_4); lemma (lm); palea (pa); stamen (st). (164x).

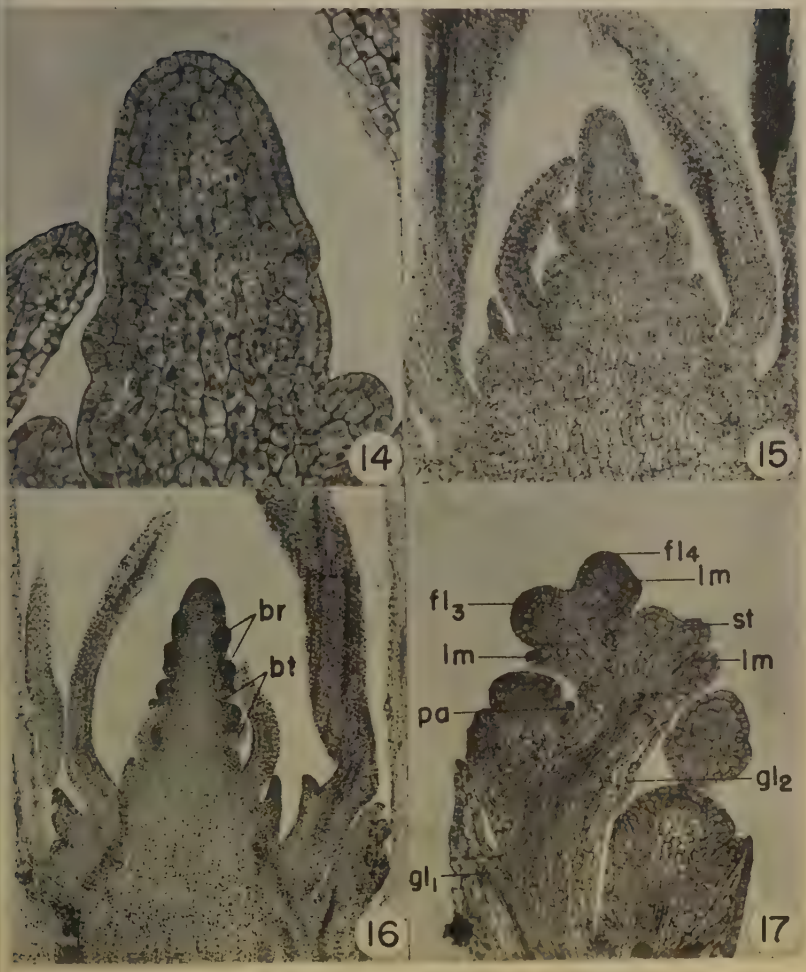
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PLATE IV

Figs. 18-20. Diagrams to show histogenesis in the branch (br) and bract (bt) during the transition of the shoot apex of Dactylis glomerata L.

Fig. 18. Areas (a) and (b) enlarged in Figs. 19 and 20.

Fig. 19. Area (a) as seen in Fig. 18. The heavy lines delimit the two layers of the tunica. (215x).

Fig. 20. Area (b) as seen in Fig. 18 enlarged to show histogens. (215x).

Figs. 21-23. Diagrams of longitudinal sections of the spikelets of D. glomerata showing the mode of development and organogeny of the florets. The heavy line delimits the tunica layers. First glume (gl_1); second glume (gl_2); lemma (lm); first floret (fl_1); second floret (fl_2). (215x).

Fig. 21. Glume initiation.

Fig. 22. Initiation of successive florets, and organogeny with the floret.

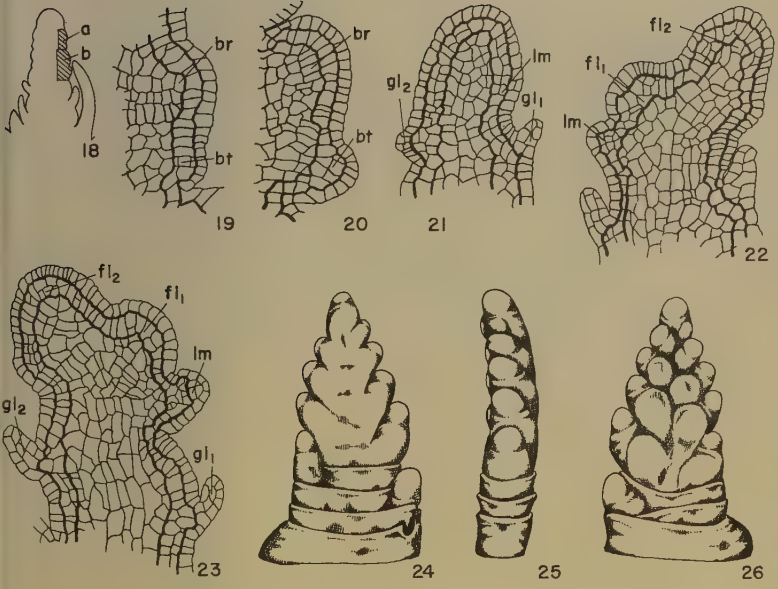
Fig. 23. Older spikelet with two floret primordia.

Figs. 24-26. Inflorescence of D. glomerata at the time of second-order branch initiation. (50x).

Fig. 24. Back view of an inflorescence.

Fig. 25. Side view of an inflorescence. Note that the apex becomes slightly curved.

Fig. 26. Face view of an inflorescence.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